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(54) **INFLUENZA HEMAGGLUTININ AND NEURAMINIDASE VARIANTS**

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(57) **ABSTRACT**

Polypeptides, polynucleotides, methods, compositions, and vaccines comprising (avian pandemic) influenza hemagglutinin and neuraminidase variants are provided.

22 Claims, 31 Drawing Sheets

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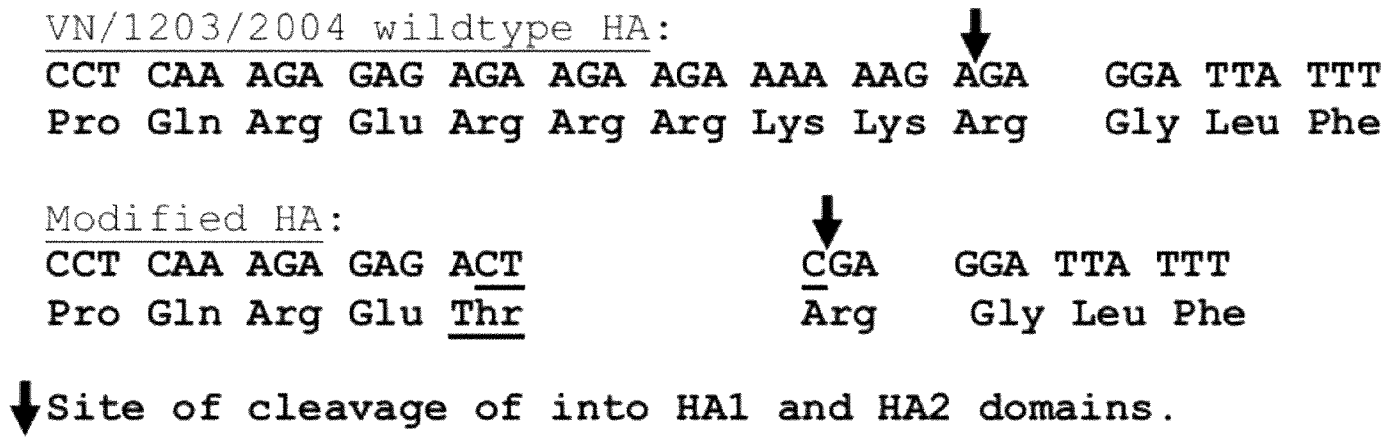


Residues that were mutagenized are underlined.

Fig 1.

Virus	Mortality (dead/total)	Virus isolation from swabs					Antibody detected/ total
		Oropharyngeal			Cloacal		
		# shedding/ total	Mean $\log_{10}$ titer (EID_{50})		# shedding/ total	Mean $\log_{10}$ titer (EID_{50})	
1997, 2003 and 2004 H5N1 wt	8/8	8/8	>6.3		8/8	>4.5	NA
1997, 2003 and 2004 H5N1 ca	0/8	0/8	<0.9		0/8	<0.9	0/8

Chickens were inoculated intranasally with 10^6 $TCID_{50}$ of virus.

Fig 2.

	LD_{50} in mice
A/AA/6/60 ca	$>10^7$ $TCID_{50}$
A/HK/491/97	10^2 $TCID_{50}$
1997 H5N1/AA ca	$>10^7$ $TCID_{50}$
A/HK/213/2003	10^6 $TCID_{50}$
2003 H5N1/AA ca	$>10^7$ $TCID_{50}$
A/Vietnam/1203/2004	$10^{0.4}$ $TCID_{50}$
2004 H5N1/AA ca	$>10^7$ $TCID_{50}$

Fig 3.

Tissue	Virus	Average fold difference in titer Over 3 days
LUNGS	A/AA/6/60	93
	1997 H5N1	501
	2003 H5N1	12
	2004 H5N1	430
NASAL TURBINATES	A/AA/6/60	32
	1997 H5N1	185
	2003 H5N1	none
	2004 H5N1	100

10^6 $TCID_{50}$ of virus was administered intranasally and tissues were harvested on days 2, 3 or 4 post-infection. Virus titers are expressed as $\log_{10}$ $TCID_{50}$/g of tissue.

Fig 4.

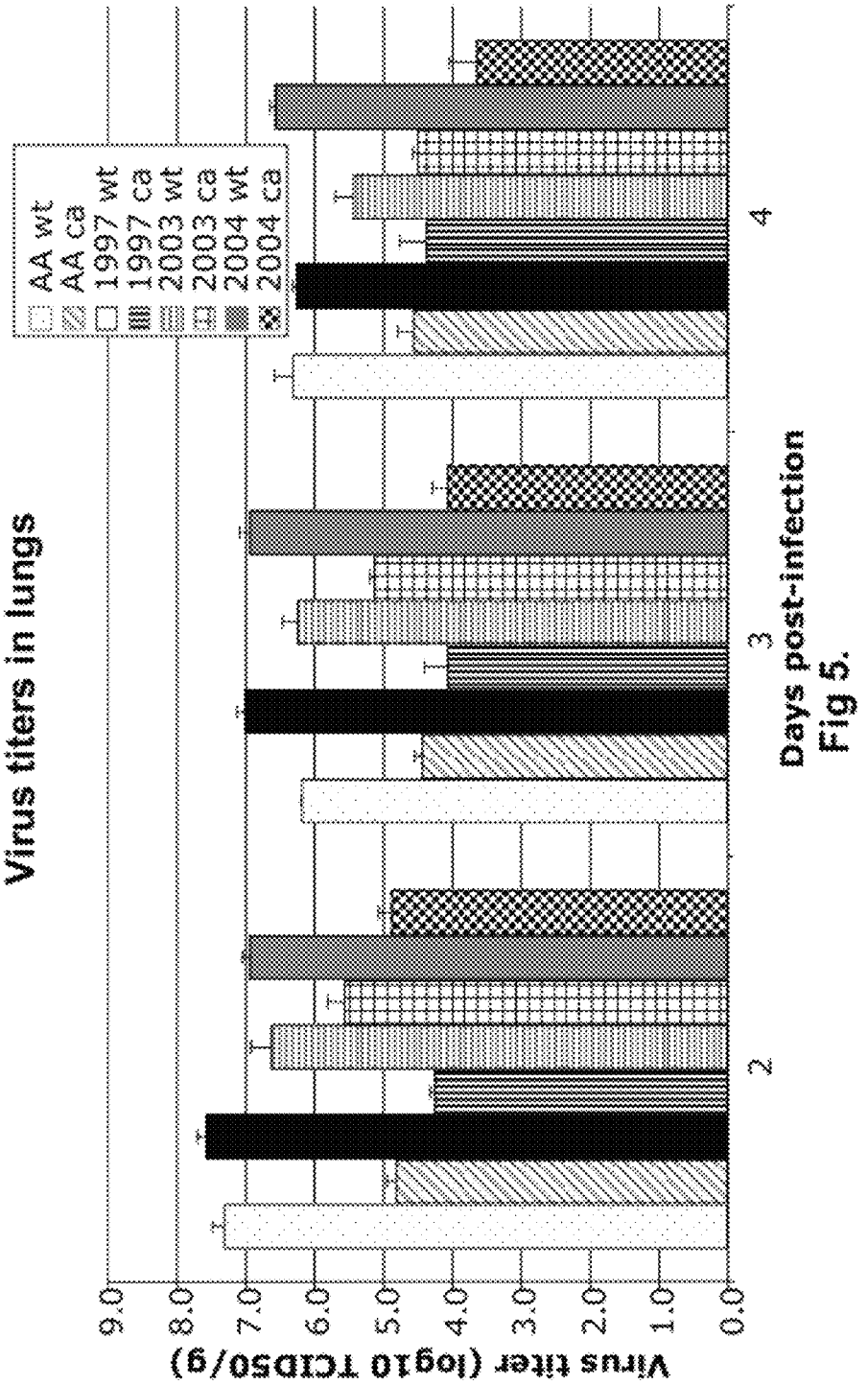

Virus titers in lungs
AA wt
AA ca
1997 wt
1997 ca
2003 wt
2003 ca
2004 wt
2004 ca
Virus titer (log10 TCID50/g)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2
3
4
Days post-infection

Fig 5.

Immunizing virus	Geometric mean serum HAI Ab titers against indicated virus		
	1997 wt	2003 wt	2004 wt
2003 ca	20	213.6	20
2003 wt	20	394	20

An undetectable titer is assigned a value of 20

Fig 6.

Immunizing virus	Geometric mean serum neutralizing Ab titers against indicated virus		
	1997 wt	2003 wt	2004 wt
2003 ca	10	59.2	10
2003 wt	10	93.3	10

An undetectable titer is assigned a value of 10

Fig 7.

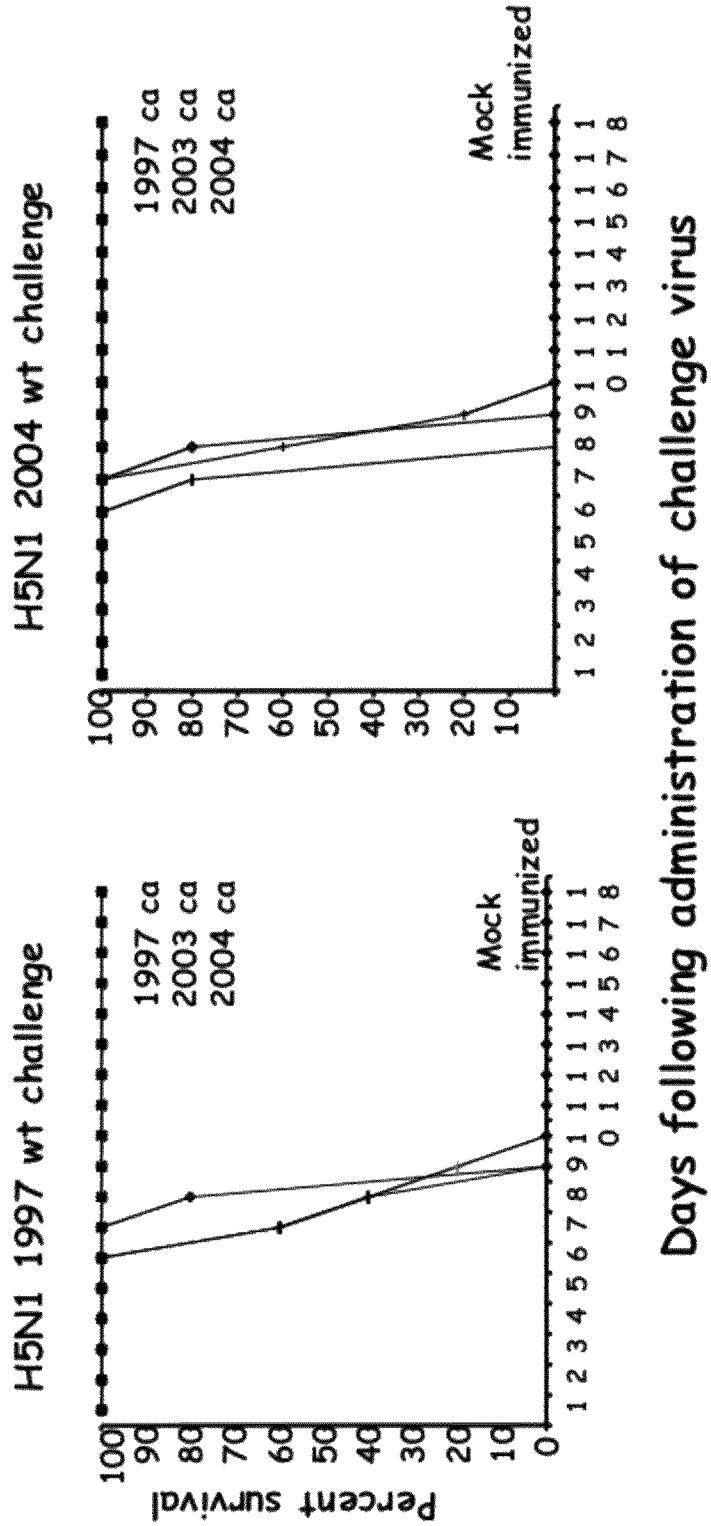

H5N1 1997 wt challenge
H5N1 2004 wt challenge
1997 ca
2003 ca
2004 ca
Mock immunized
Percent survival
Days following administration of challenge virus

Fig 8.

Immunization	Mean reduction in titer in lungs following Homologous challenge	Heterologous H5N1 challenge		
		1997 wt	2003 wt	2004 wt
1997 ca	2.5	NA	3.0	0.7
2003 ca	>5.8	2.3	NA	2.9
2004 ca	2.0	1.4	>5.7	NA

Fig 9.

Immunization	Mean reduction in titer in NT following			
	Homologous challenge	Heterologous H5N1 challenge		
		1997 wt	2003 wt	2004 wt
1997 ca	4.3	NA	>1.2	2.6
2003 ca	>1.2	3.7	NA	>3.3
2004 ca	1.6	4.2	>3.5	NA

Fig 10.

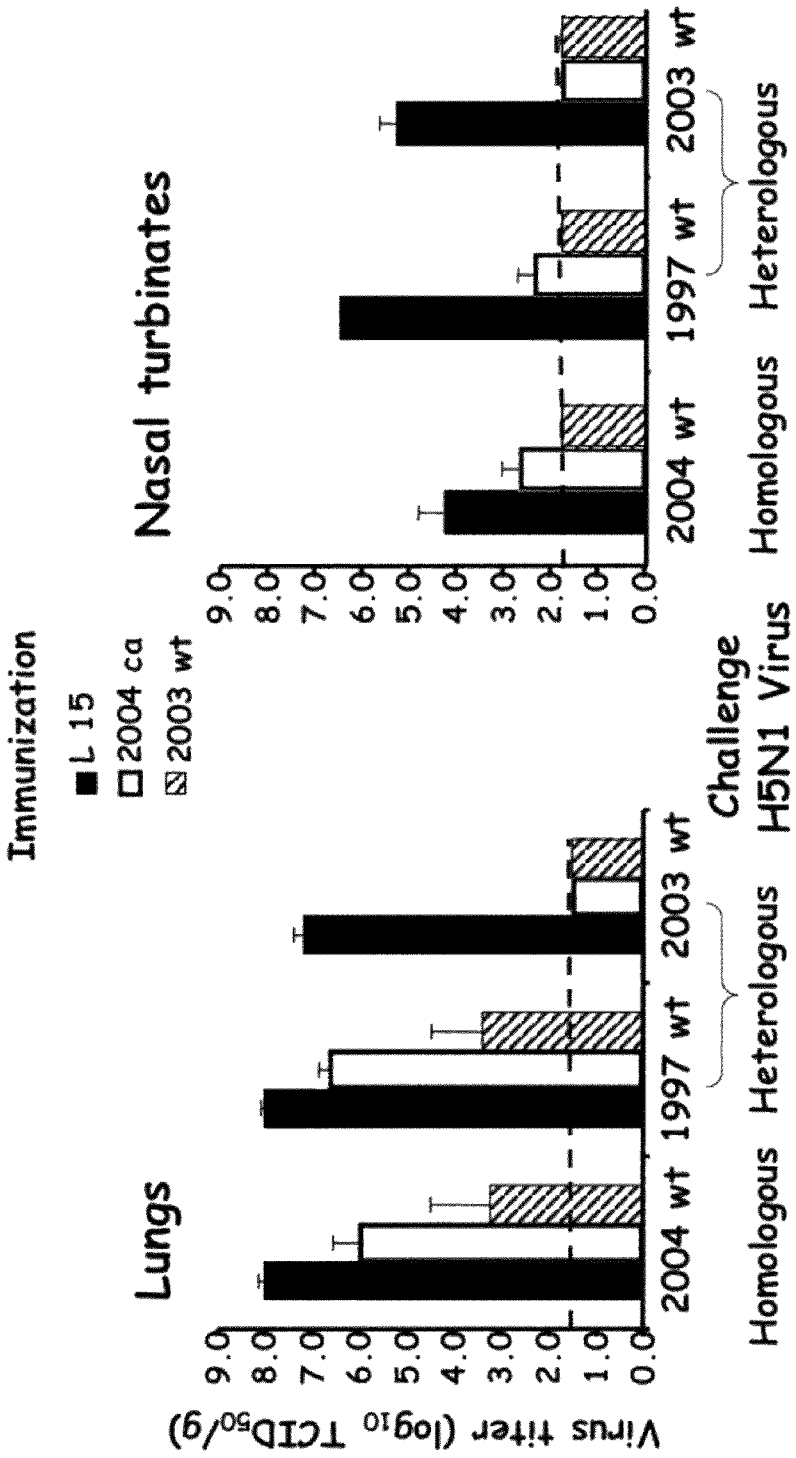

Immunization
L 15
2004 ca
2003 wt
Lungs
Nasal turbinates
Virus titer (log10 TCID50/g)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2004 wt
1997 wt
2003 wt
Homologous
Heterologous
Challenge
H5N1 Virus

Fig 11.

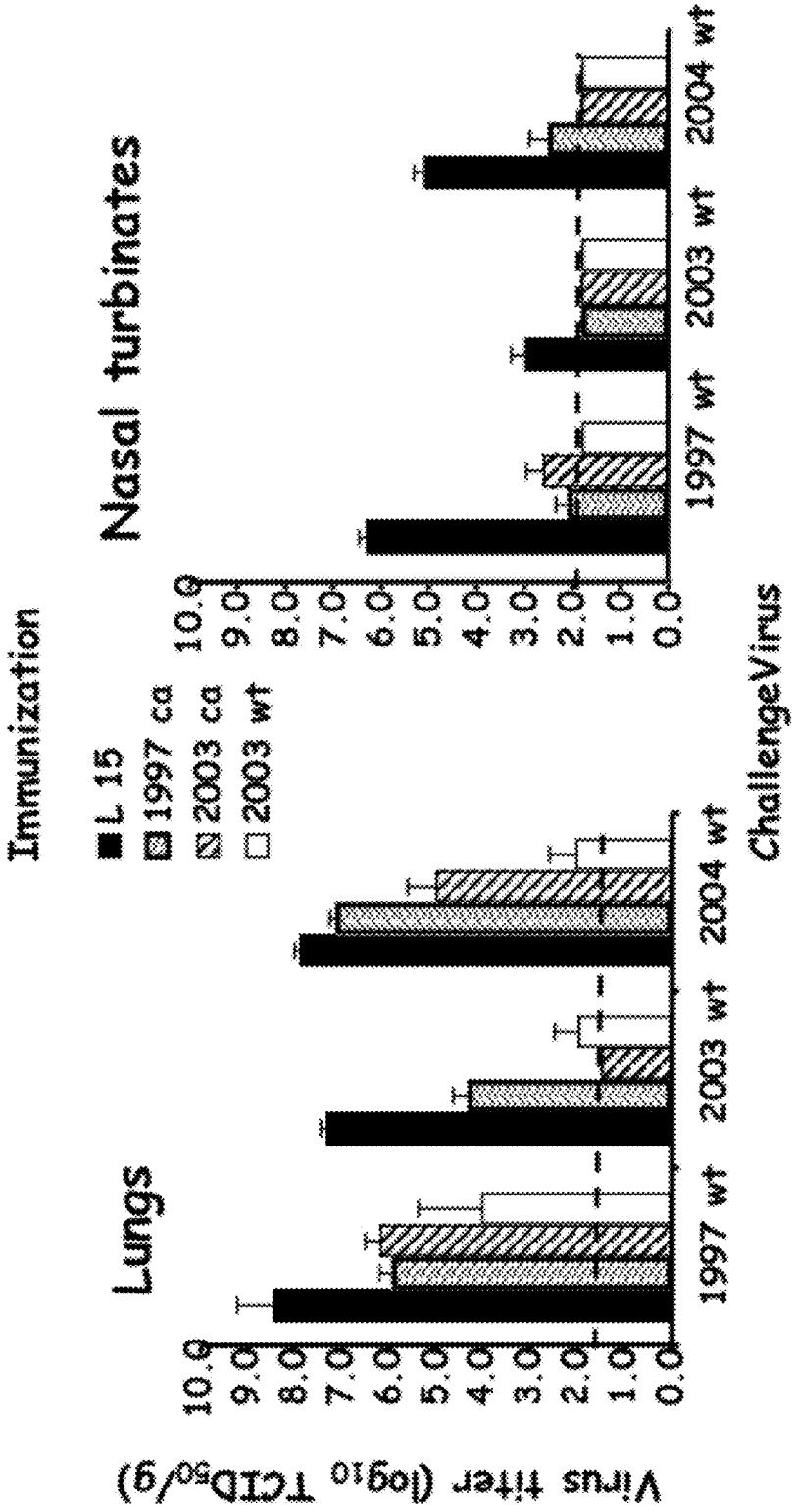

Virus titer (log_{10} $TCID_{50}$/g)
Lungs
Nasal turbinates
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1997 wt
2003 wt
2004 wt
Immunization
L 15
1997 ca
2003 ca
2003 wt
Challenge Virus

Fig 12.

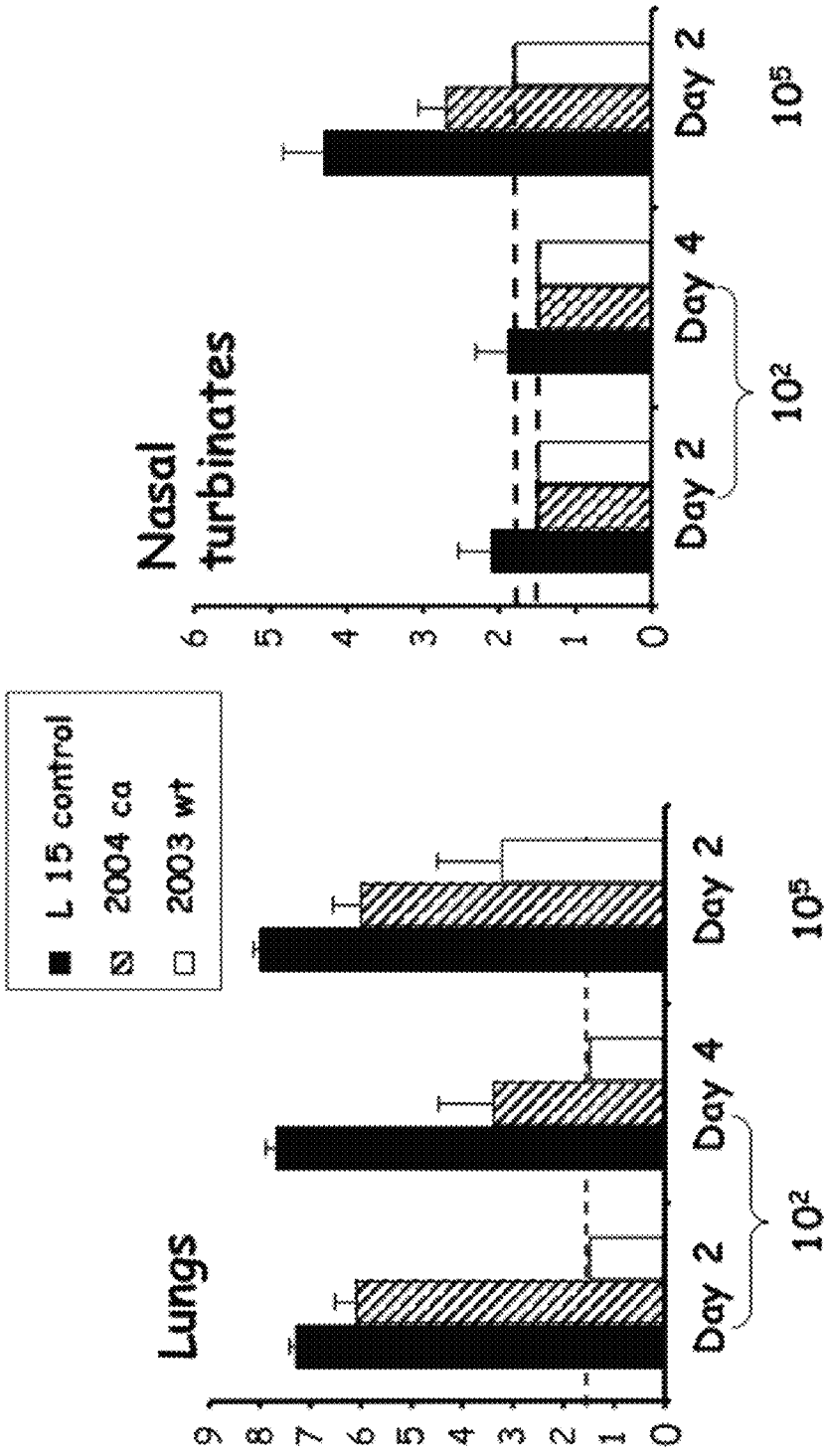

L 15 control
2004 ca
2003 wt
Lungs
Nasal turbinates
Day 2
Day 4
Day 2
10^2
Day 2
Day 4
Day 2
10^5
Challenge dose

Fig 13.

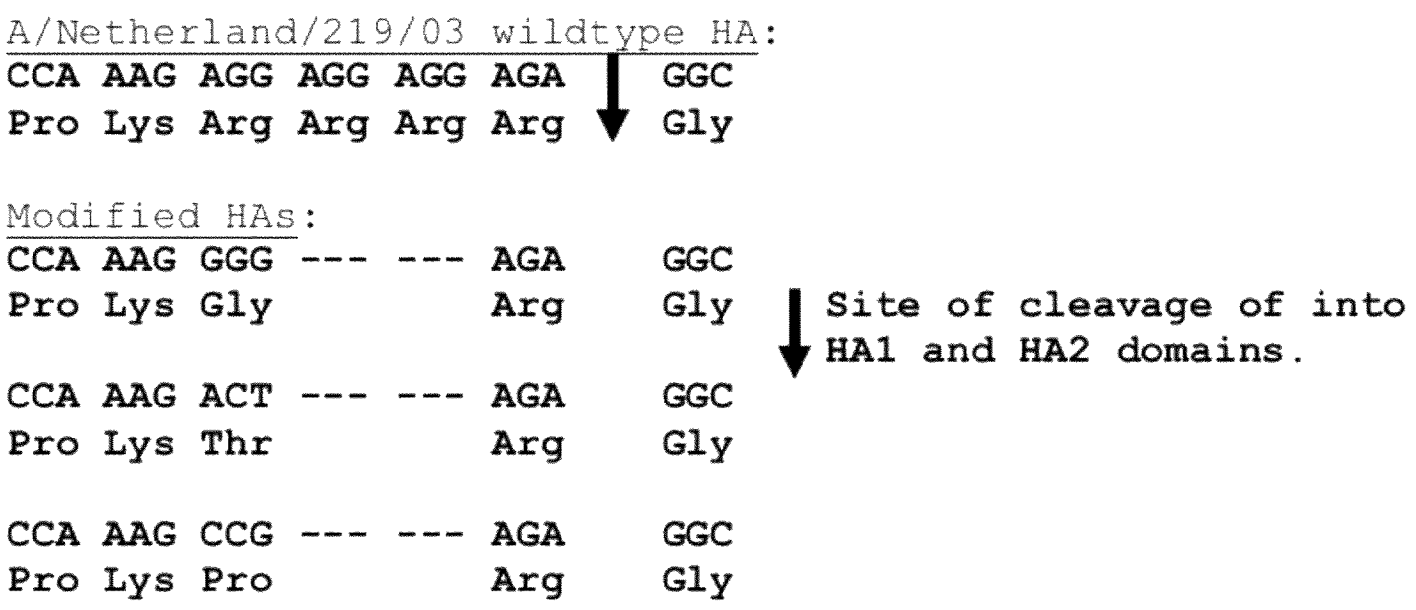

A/Netherland/219/03 wildtype HA:
CCA AAG AGG AGG AGG AGA GGC
Pro Lys Arg Arg Arg Arg Gly
Modified HAs:
CCA AAG GGG --- --- AGA GGC
Pro Lys Gly Arg Gly
Site of cleavage of into HA1 and HA2 domains.
CCA AAG ACT --- --- AGA GGC
Pro Lys Thr Arg Gly
CCA AAG CCG --- --- AGA GGC
Pro Lys Pro Arg Gly

Fig 14.

Immunizing virus	Doses	Geometric mean serum neutralizing Ab titers against indicated virus		
		1997 wt	2003 wt	2004 wt
A/VN/2004 *ca*	1	10	10	10
	2	160	528	388
A/HK/2003 *ca*	1	10	37	10
	2	19	1056	61

Fig 15.

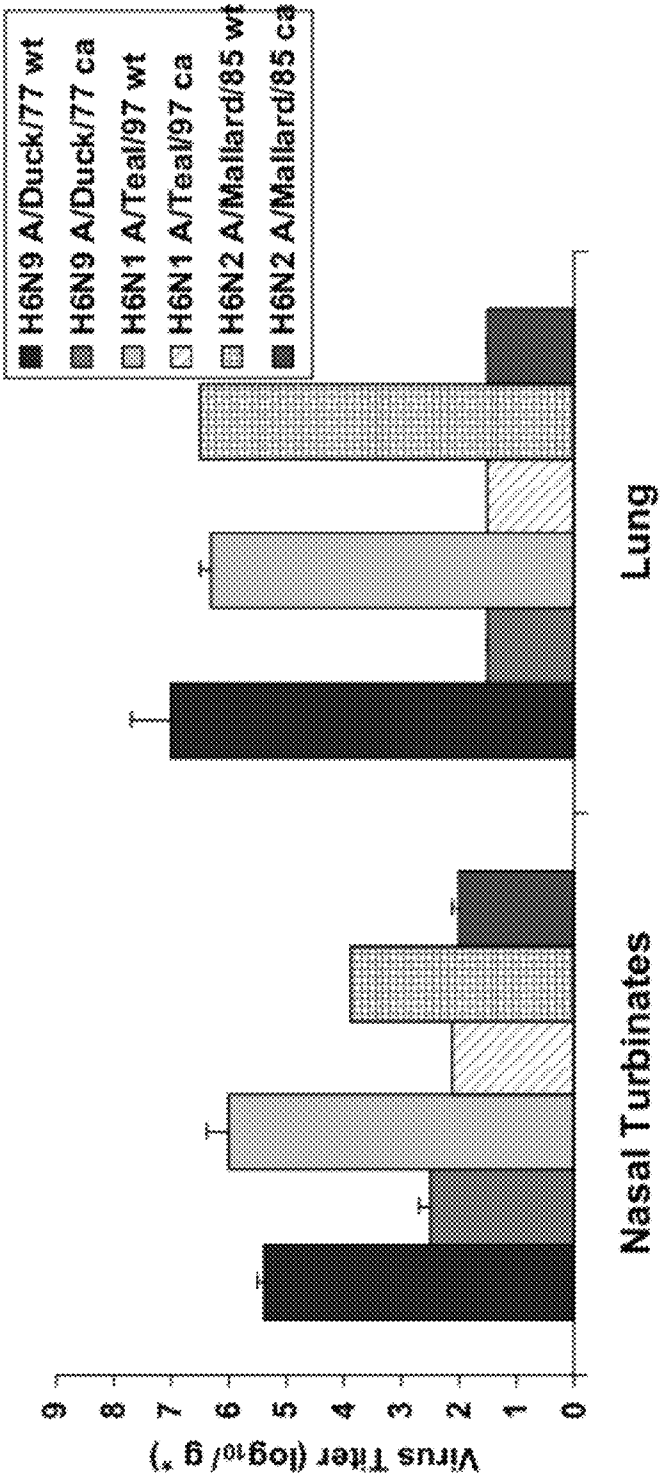
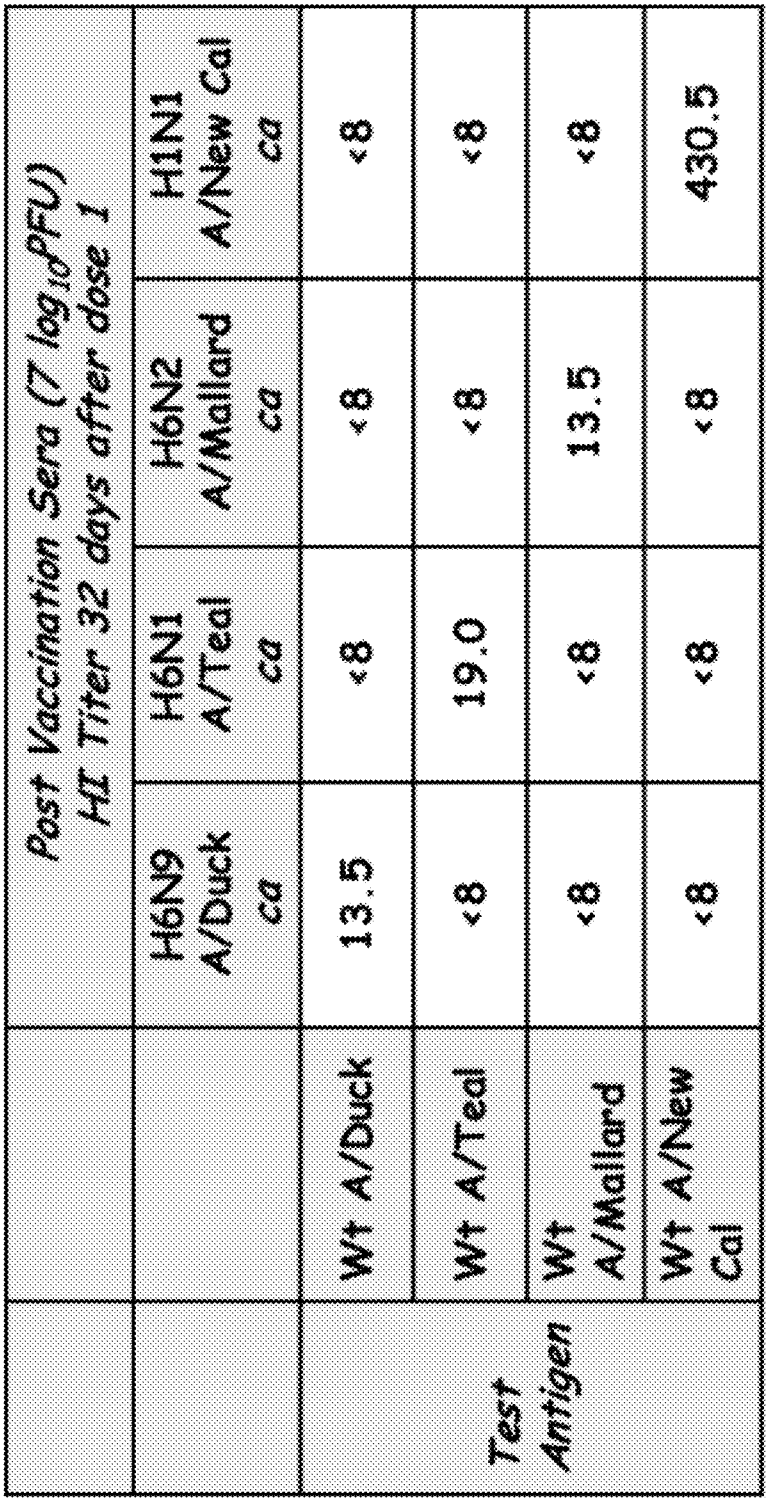

H6N9 A/Duck/77 wt
H6N9 A/Duck/77 ca
H6N1 A/Teal/97 wt
H6N1 A/Teal/97 ca
H6N2 A/Mallard/85 wt
H6N2 A/Mallard/85 ca
Virus Titer (log10/ g *)
0
1
2
3
4
5
6
7
8
9
Nasal Turbinates
Lung

Fig 16.



		Post Vaccination Sera (7 log_{10}PFU) HI Titer 32 days after dose 1			
		H6N9 A/Duck ca	H6N1 A/Teal ca	H6N2 A/Mallard ca	H1N1 A/New Cal ca
Test Antigen	Wt A/Duck	13.5	<8	<8	<8
	Wt A/Teal	<8	19.0	<8	<8
	Wt A/Mallard	<8	<8	13.5	<8
	Wt A/New Cal	<8	<8	<8	430.5

Fig 17.

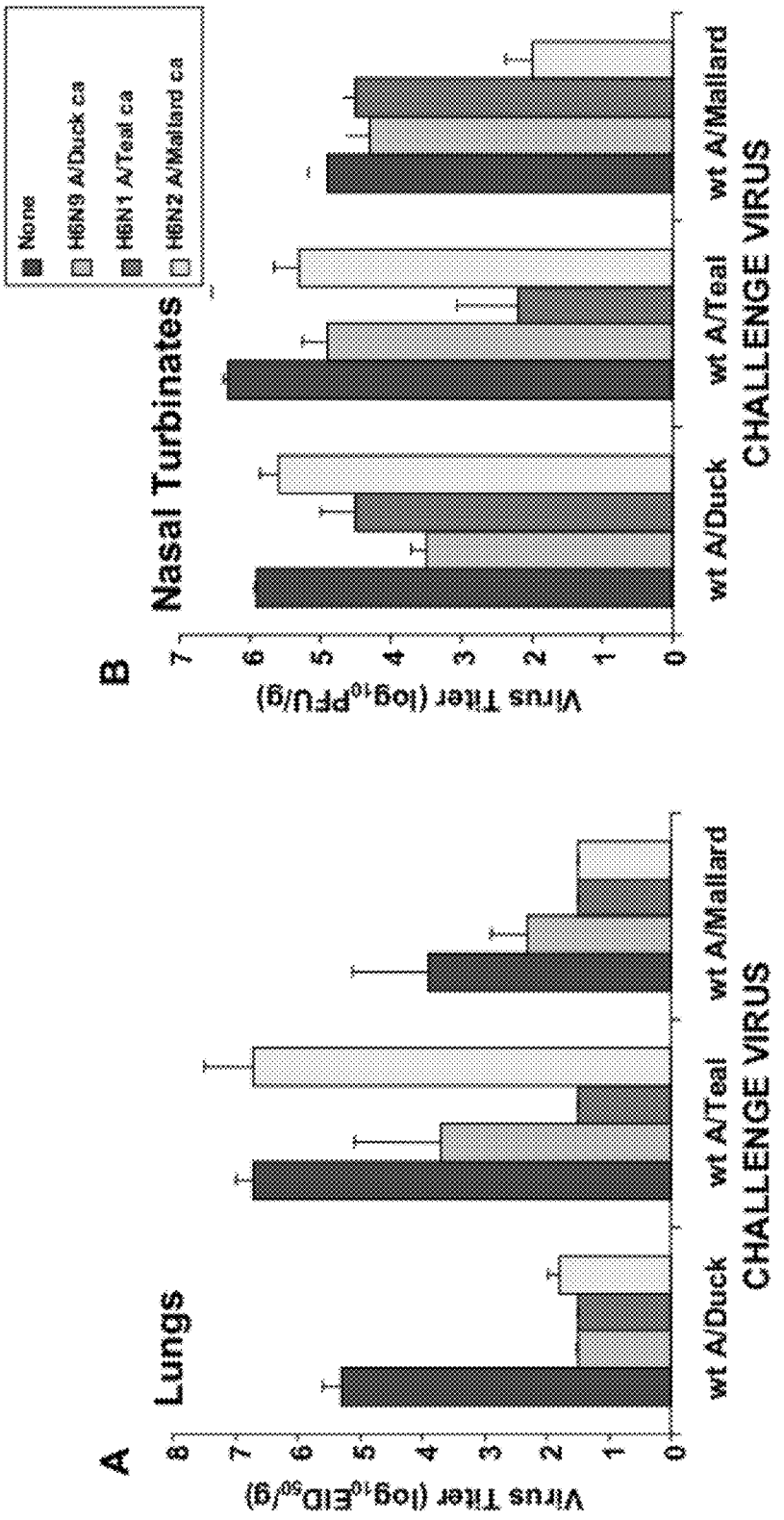

A
Lungs
Virus Titer (log10EID50/g)
0
1
2
3
4
5
6
7
8
wt A/Duck
wt A/Teal
wt A/Mallard
CHALLENGE VIRUS
B
Nasal Turbinates
Virus Titer (log10PFU/g)
0
1
2
3
4
5
6
7
None
H6N9 A/Duck ca
H6N1 A/Teal ca
H6N2 A/Mallard ca
wt A/Duck
wt A/Teal
wt A/Mallard
CHALLENGE VIRUS

Fig 18.AB

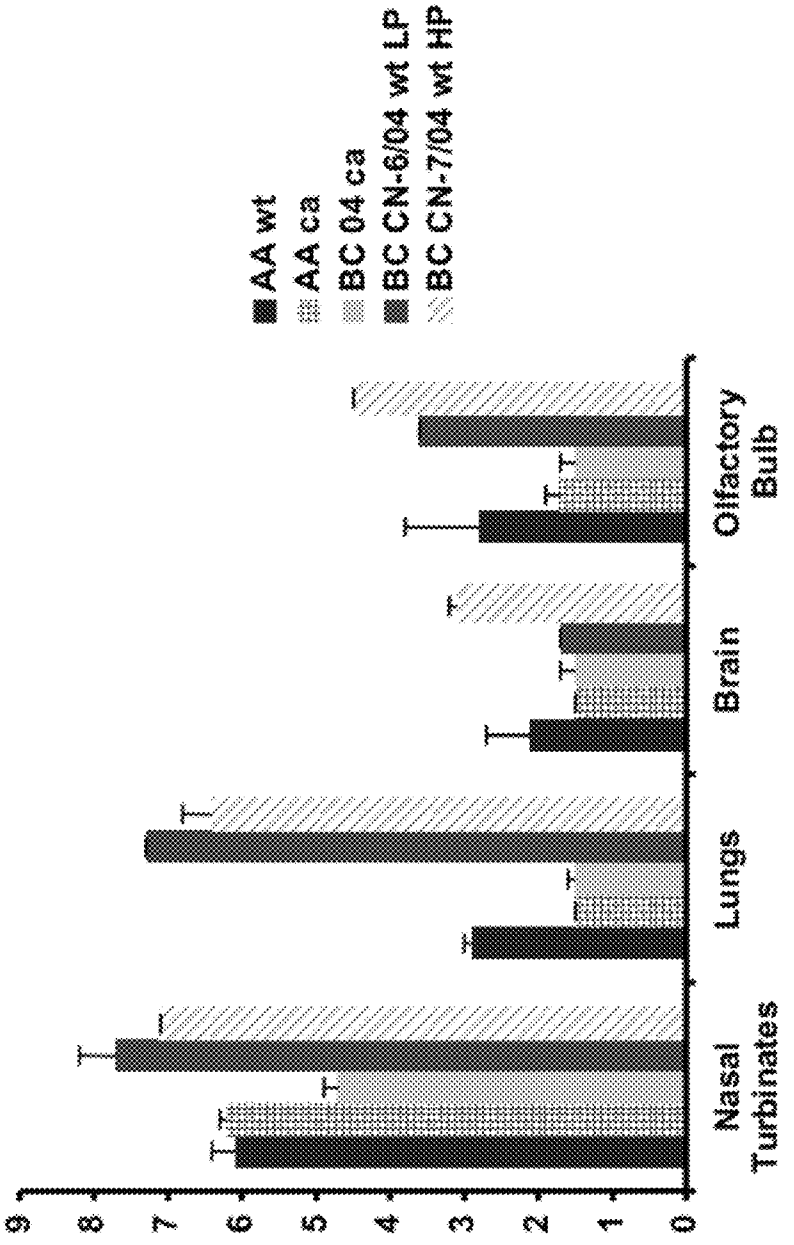

AA wt
AA ca
BC 04 ca
BC CN-6/04 wt LP
BC CN-7/04 wt HP
9
8
7
6
5
4
3
2
1
0
Nasal Turbinates
Lungs
Brain
Olfactory Bulb

Fig 19.

Virus	Dosing schedule	Geometric mean neutralizing antibody titers achieved at indicated time post-infection[a]		
		d 0	4 weeks	8 weeks
H7N3 BC 04 ca	d 0	<10	80	NA
	d 0	<10	87	403[b]
	d 0 and d 28	<10	45	470
A/ck/BC/CN-6/04	d 0	<10	308	NA
	d 0	<10	320	941
	d 0 and d 28	<10	154	1701

Fig 20.

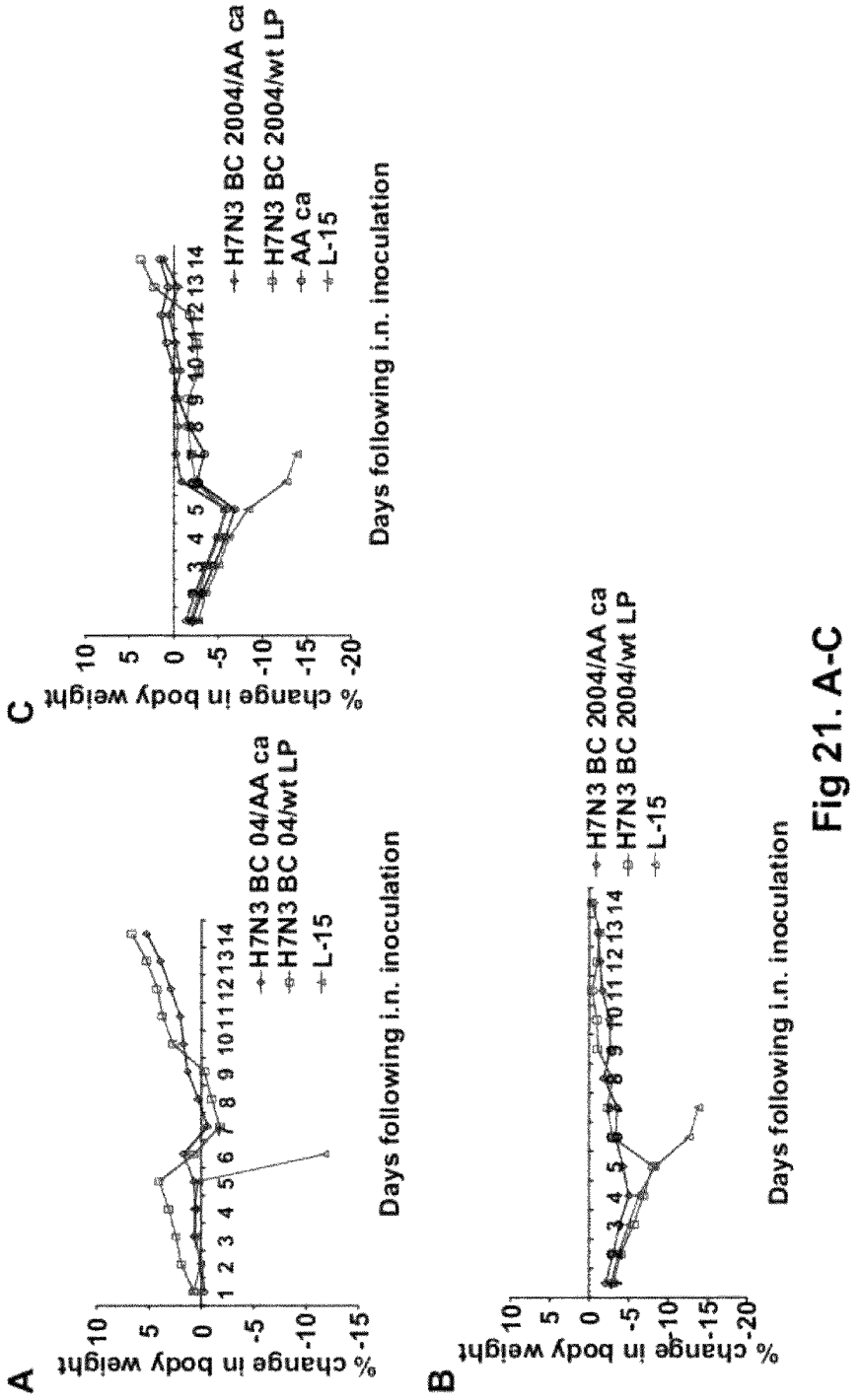
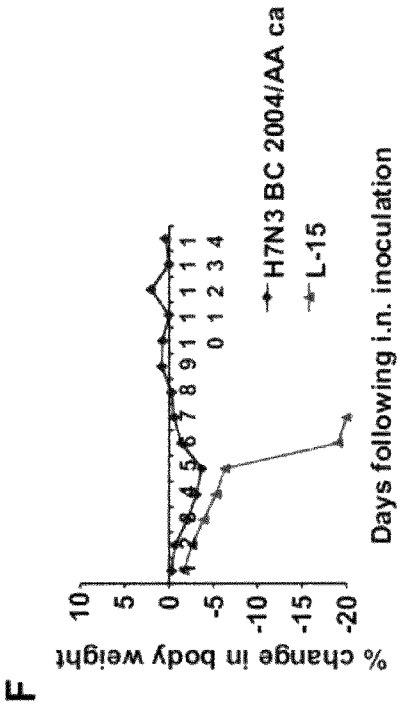
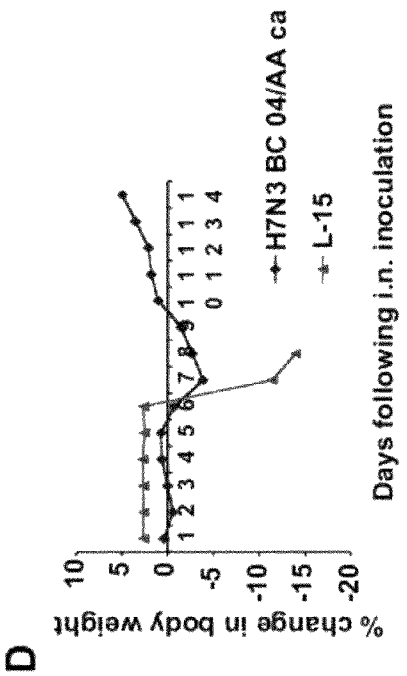

A
% change in body weight
10
5
0
-5
-10
-15
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days following i.n. inoculation
H7N3 BC 04/AA ca
H7N3 BC 04/wt LP
L-15
B
% change in body weight
10
5
0
-5
-10
-15
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days following i.n. inoculation
H7N3 BC 2004/AA ca
H7N3 BC 2004/wt LP
L-15
C
% change in body weight
10
5
0
-5
-10
-15
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days following i.n. inoculation
H7N3 BC 2004/AA ca
H7N3 BC 2004/wt LP
AA ca
L-15

Fig 21. A-C


F
% change in body weight
10
5
0
-5
-10
-15
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14
H7N3 BC 2004/AA ca
L-15
Days following i.n. inoculation


D
% change in body weight
10
5
0
-5
-10
-15
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14
H7N3 BC 04/AA ca
L-15
Days following i.n. inoculation

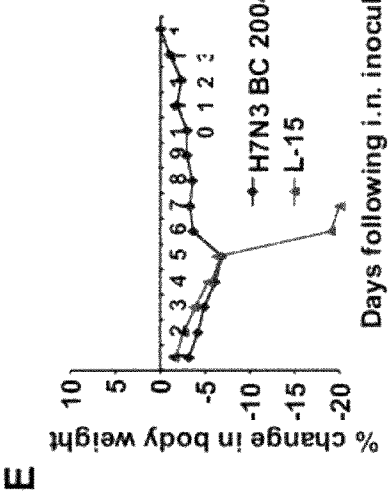
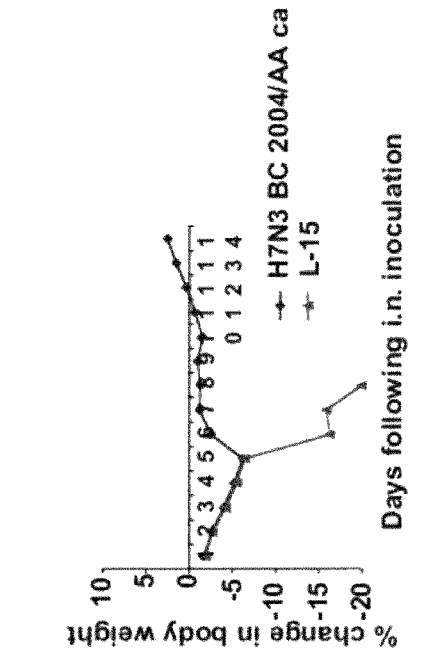
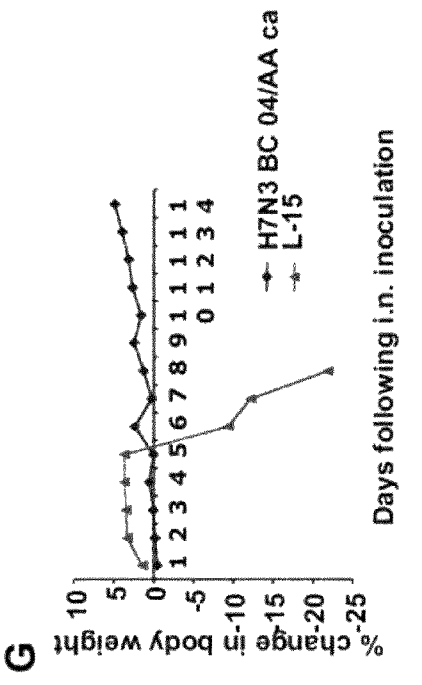

E
% change in body weight
10
5
0
-5
-10
-15
-20
2 3 4 5 6 7 8 9 10 11 12 13
H7N3 BC 2004/AA ca
L-15
Days following i.n. inoculation

Fig 21. D-F


I
% change in body weight
10
5
0
-5
-10
-15
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14
H7N3 BC 2004/AA ca
L-15
Days following i.n. inoculation


G
% change in body weight
10
5
0
-5
-10
-15
-20
-25
1 2 3 4 5 6 7 8 9 10 11 12 13 14
H7N3 BC 04/AA ca
L-15
Days following i.n. inoculation

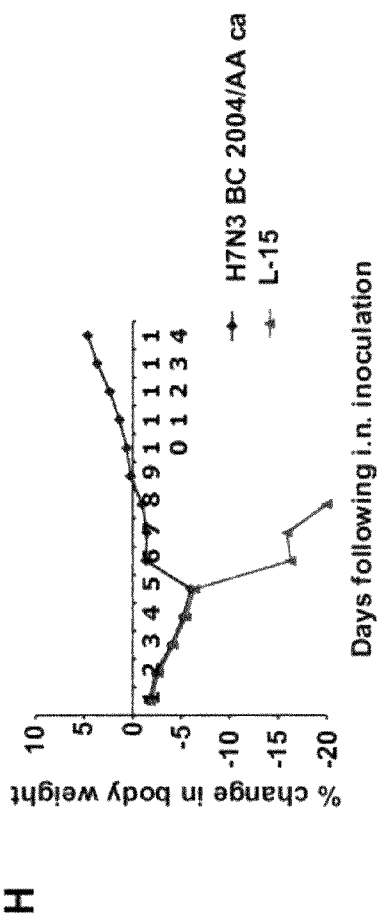

H
% change in body weight
10
5
0
-5
-10
-15
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14
H7N3 BC 2004/AA ca
L-15
Days following i.n. inoculation

Fig 21. G-I

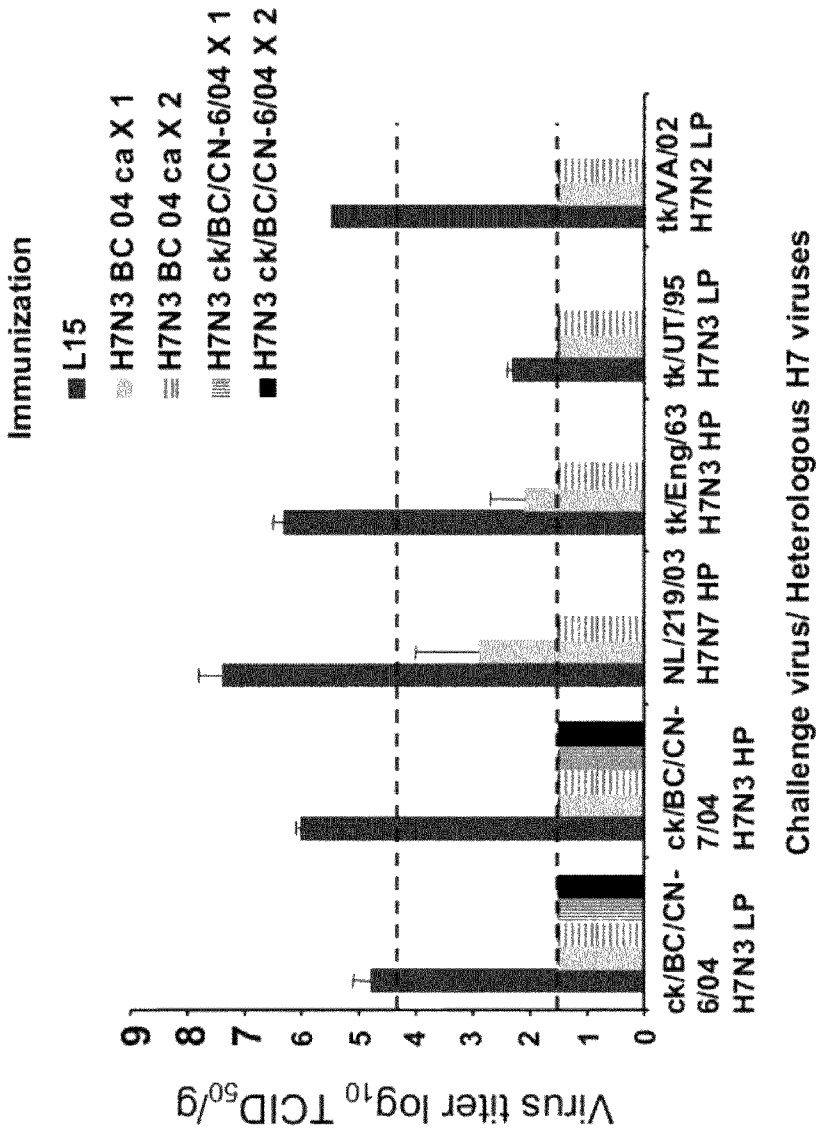

Immunization
L15
H7N3 BC 04 ca X 1
H7N3 BC 04 ca X 2
H7N3 ck/BC/CN-6/04 X 1
H7N3 ck/BC/CN-6/04 X 2
Virus titer log10 TCID50/g
0
1
2
3
4
5
6
7
8
9
ck/BC/CN-6/04 H7N3 LP
ck/BC/CN-7/04 H7N3 HP
NL/219/03 H7N7 HP
tk/Eng/63 H7N3 HP
tk/UT/95 H7N3 LP
tk/VA/02 H7N2 LP
Challenge virus/ Heterologous H7 viruses

Fig 22.A

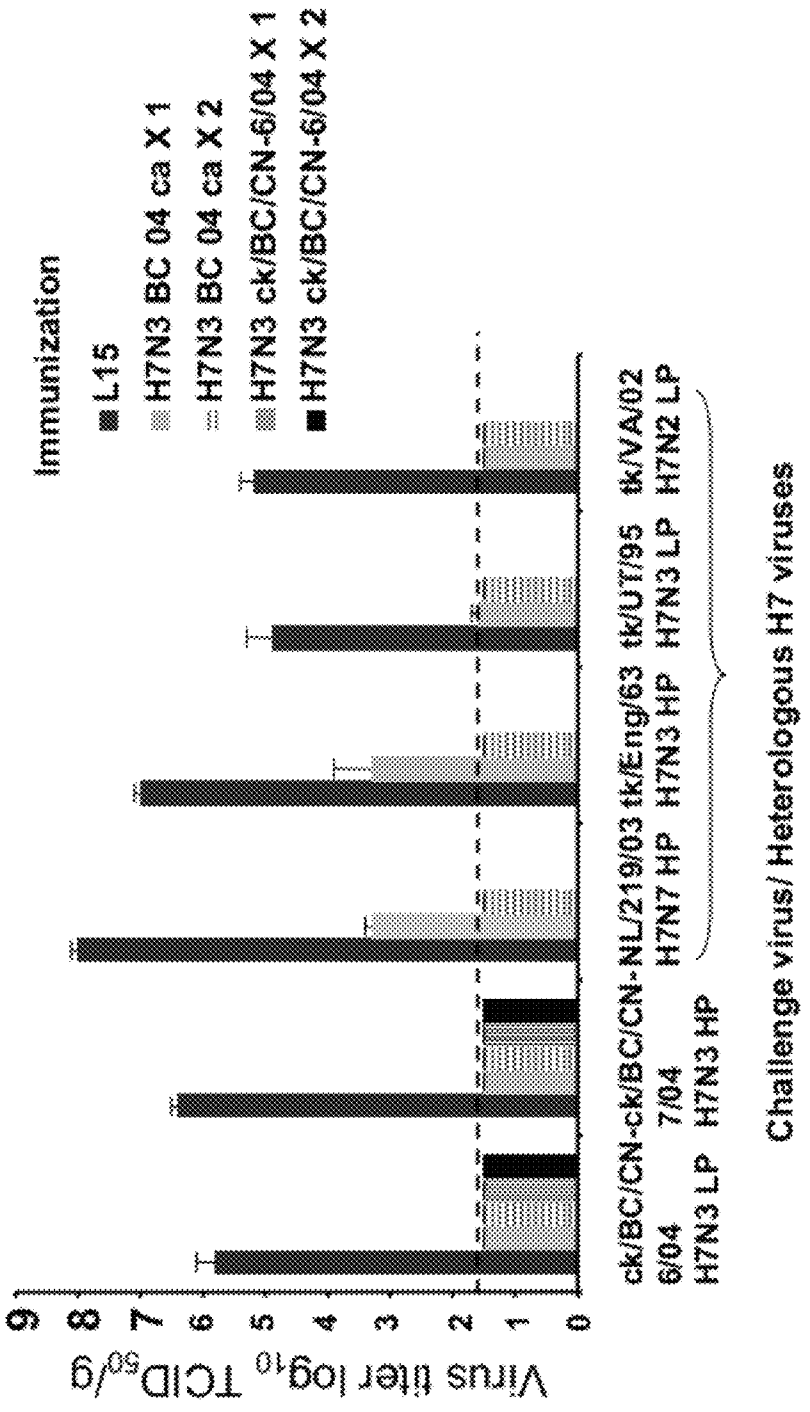

Immunization
L15
H7N3 BC 04 ca X 1
H7N3 BC 04 ca X 2
H7N3 ck/BC/CN-6/04 X 1
H7N3 ck/BC/CN-6/04 X 2
Virus titer log10 TCID50/g
0
1
2
3
4
5
6
7
8
9
ck/BC/CN-ck/BC/CN-NL/219/03 tk/Eng/63 tk/UT/95 tk/VA/02
6/04 7/04 H7N7 HP H7N3 HP H7N3 LP H7N2 LP
H7N3 LP H7N3 HP
Challenge virus/ Heterologous H7 viruses

Fig 22.B

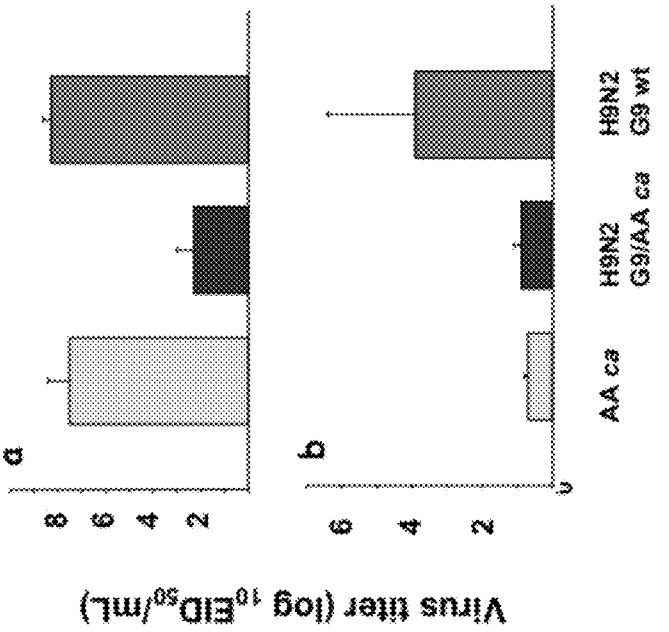

a
b
8
6
4
2
6
4
2
Virus titer (log $_{10}$EID$_{50}$/mL)
AA ca
H9N2 G9/AA ca
H9N2 G9 wt
Virus

Fig 23.

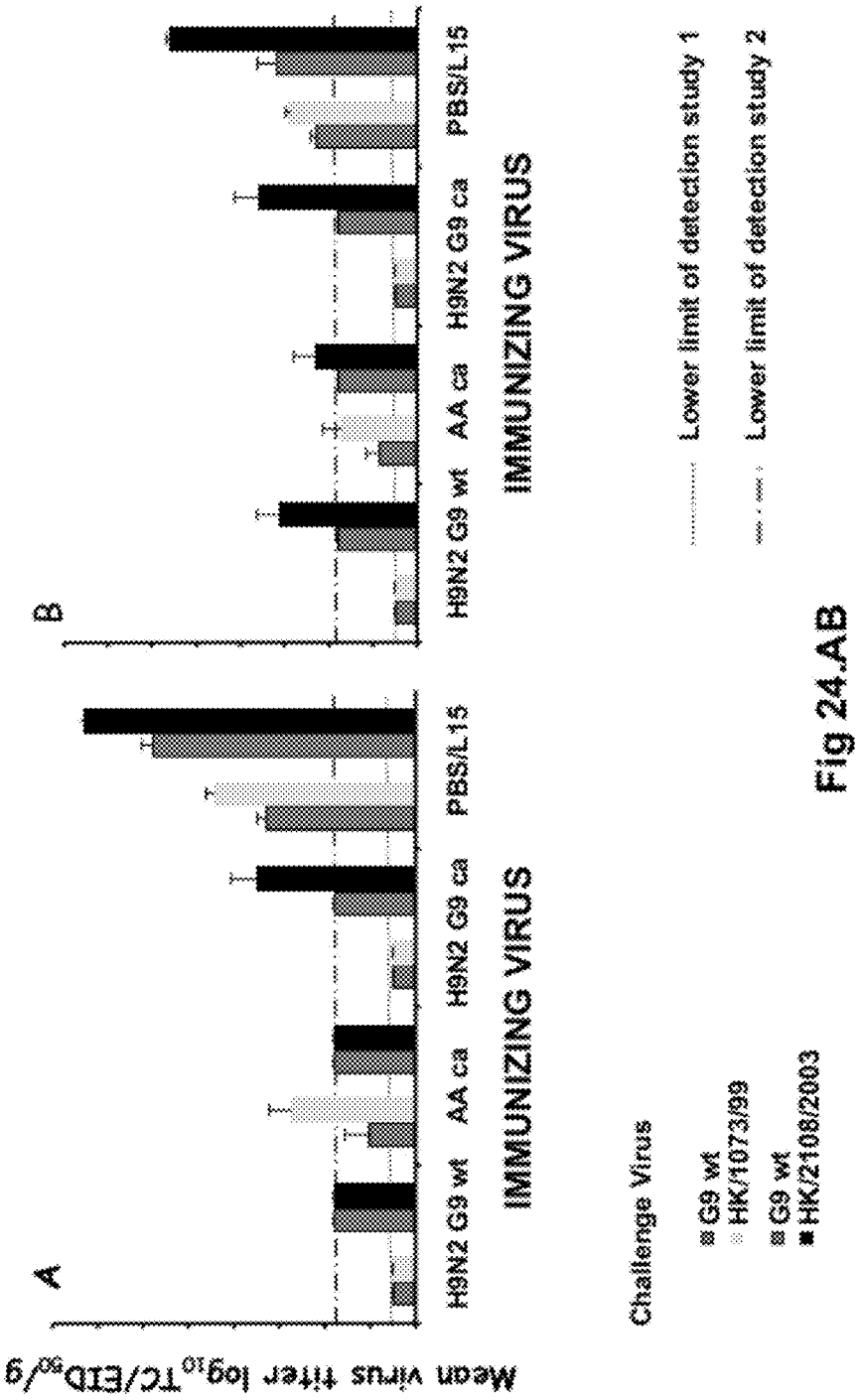

A
B
Mean virus titer $log_{10}TC/EID_{50}/g$
H9N2 G9 wt
AA ca
H9N2 G9 ca
PBS/L15
IMMUNIZING VIRUS
Challenge Virus
G9 wt HK/1073/99
G9 wt HK/2108/2003
Lower limit of detection study 1
Lower limit of detection study 2

Fig 24.AB

Vaccine Dose	N	No. Vaccine Virus Culture + (days)	Peak Titer Shed* ($log_{10}TCID_{50}$/mL)	No. Vaccine Virus PCR+ (days)
1	26	2 (1)	1.0	8 (1.1)
2	24	0	$\leq$0.6	2 (1.5)

* Among those who shed virus

Fig 25.

Vaccine Dose	Sero-status	N	Mean HI titer, $1/\log_2$ Days After Dose D0	D28	D42	No. with 4-fold rise	%
1	-	26	1.7	2.6	- -	9	31
2	-	24	3.1	4.6	4.5	12	50
Cumulative		24				22	92

Fig 26.

Vaccine Dose	N	No. Nasal wash Culture + (days)	No. Nasal wash PCR+ (days)
1	21	0	2 (2.0)
2	18	0	3 (1.0)

Vaccine virus was not detected by any method in throat swab specimens.

Fig 27.

Vaccine Dose	N	Mean HI titer, $1/\log_2$				
		Days after dose				
		D0	D28	No. with 4-fold rise	%
1	20	2.6	2.8	0	0
2	18	2.7	3.1	1	6
Cumulative	18			2	11

Fig 28.

INFLUENZA HEMAGGLUTININ AND NEURAMINIDASE VARIANTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 11/836,369, filed Aug. 9, 2007, now U.S. Pat. No. 8,039,002, and claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application nos. 60/821,832 filed Aug. 9, 2006 and 60/942,804, filed Jun. 8, 2007, the disclosures of each of which are incorporated herein in their entirety for all purposes.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Apr. 26, 2011, is named MDI-0438-UT.txt and is 151,676 bytes in size.

BACKGROUND OF THE INVENTION

Vaccines against various and evolving strains of influenza are important from a community health stand point, as well as commercially, since each year numerous individuals are infected with different strains and types of influenza virus. Infants, the elderly, those without adequate health care and immuno-compromised persons are at special risk of death from such infections. Compounding the problem of influenza infections is that novel influenza strains evolve readily and can spread amongst various species, thereby necessitating the continuous production of new vaccines.

Numerous vaccines capable of producing a protective immune response specific for such different and influenza viruses/virus strains have been produced for over 50 years and include whole virus vaccines, split virus vaccines, surface antigen vaccines and live attenuated virus vaccines. However, while appropriate formulations of any of these vaccine types are capable of producing a systemic immune response, live attenuated virus vaccines have the advantage of also being able to stimulate local mucosal immunity in the respiratory tract. Considerable work in the production of influenza viruses, and fragments thereof, for production of vaccines has been done by the present inventors and co-workers; see, e.g., U.S. Application Nos. 60/420,708, filed Oct. 23, 2002; 60/574,117, filed May 24, 2004; 10/423,828, filed Apr. 25, 2003; 60/578,962, filed Jun. 12, 2004; and 10/870,690 filed Jun. 16, 2004, the disclosure of which is incorporated by reference herein.

Because of the continual emergence (or re-emergence) of different influenza strains, new influenza vaccines are continually desired. Such vaccines typically are created using antigenic moieties of the newly emergent virus strains, thus, polypeptides and polynucleotides of novel, newly emergent, or newly re-emergent virus strains (especially sequences of antigenic genes) are highly desirable.

The present invention provides new and/or newly isolated influenza hemagglutinin and neuraminidase variants that are capable of use in production of numerous types of vaccines as well as in research, diagnostics, etc. Numerous other benefits will become apparent upon review of the following.

SUMMARY OF THE INVENTION

In some aspects herein, the invention comprises an isolated or recombinant polypeptide that is selected from: any one of the polypeptides encoded by SEQ ID NO:1 through SEQ ID NO:10 or SEQ ID NO:21 through SEQ ID NO:26 or SEQ ID NO:33 through SEQ ID NO:38; any one of the polypeptides of SEQ ID NO:11 through SEQ ID NO:20 or SEQ ID NO:27 through SEQ ID NO:32 or SEQ ID NO:39 through SEQ ID NO:44; only the open reading frame encoding the polypeptides of SEQ ID NO:11 through SEQ ID NO:20 or SEQ ID NO:27 through SEQ ID NO:32 or SEQ ID NO:39 through SEQ ID NO:44; any alternative (e.g., the mature form without the signal peptide, or without the 5' and 3' sequences outside of the open reading frame, or the sequences as expressed on the surface of a virus (e.g., influenza)) form of the polypeptides of SEQ ID NO:11-20 or SEQ ID NO:27-32 or SEQ ID NO:39-44; any polypeptide that is encoded by a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of a polynucleotide sequence of SEQ ID NO:1 through SEQ ID NO:10 or SEQ ID NO:21 through SEQ ID NO:26, SEQ ID NO:33-38, or SEQ ID NO:45; any polypeptide that is encoded by a polynucleotide sequence which hybridizes under highly stringent conditions to a polynucleotide sequence of SEQ ID NO:1 through SEQ ID NO:10 or SEQ ID NO:21 through SEQ ID NO:26 or SEQ ID NO:33 through SEQ ID NO:38, or SEQ ID NO:45; and, a fragment of any of the above wherein the sequence comprises a hemagglutinin or neuraminidase polypeptide, or a fragment of a hemagglutinin or neuraminidase polypeptide, preferably where the fragments generate an antibody that specifically binds a full length polypeptide of the invention. In various embodiments, the isolated or recombinant polypeptides of the invention are substantially identical to about 300 contiguous amino acid residues of any of the above polypeptides. In yet other embodiments, the invention comprises isolated or recombinant polypeptides, that comprise an amino acid sequence that is substantially identical over at least about 350 amino acids; over at least about 400 amino acids; over at least about 450 amino acids; over at least about 500 amino acids; over at least about 520 amino acids; over at least about 550 amino acids; over at least about 559 amino acids; over at least about 565 amino acids; or over at least about 566 amino acids contiguous of any of the above polypeptides. In some embodiments, the polypeptide sequence (e.g., as listed in "SEQUENCES" herein) comprises less than 565, 559, etc amino acids. In such embodiments, the shorter listed polypeptides optionally comprise less than 565, 559, etc. amino acids. In yet other embodiments, the polypeptides of the invention optionally comprise fusion proteins, proteins with a leader sequence, a precursor polypeptide, proteins with a secretion signal or a localization signal, or proteins with an epitope tag, an E-tag, or a His epitope tag. In still other embodiments, the invention comprises a polypeptide comprising a sequence having at least 85%, at least 90%, at least 93%, at least 95%, at least 98%, at least 98.5%, at least 99%, at least 99.2%, at least 99.4%, at least 99.6%, at least 99.8%, or at least 99.9% sequence identity to at least one polypeptide listed above. The hemagglutinin sequences of the invention can comprise both those sequences with unmodified and modified polybasic cleavage sites (thereby allowing growth of the viruses in eggs). The hemagglutinin polypeptide sequences of SEQ ID NOS:11, 13, 15, 17, 19, 27, 29, 31, 39, 41, or 43 comprise the endogenous amino terminal signal peptide sequences, however, the hemagglutinin polypeptide sequences of the invention also include the mature (amino terminal signal peptide cleaved) form of the hemagglutinin polypeptides. The cleavage sites of any hemagglutinin polypeptide sequence of any influenza strain can be routinely measured or predicted using any number of methods in the art.

In other aspects, the invention comprises a composition with one or more polypeptide listed above, or fragments thereof. The invention also includes polypeptides that are specifically bound by a polyclonal antisera raised against at least 1 antigen that comprises at least one amino acid sequence described above, or a fragment thereof. Such antibodies specific for the polypeptides described above are also features of the invention. The polypeptides of the invention are optionally immunogenic.

The invention also encompasses immunogenic compositions comprising an immunologically effective amount of one or more of any of the polypeptides described above as well as methods for stimulating the immune system of an individual to produce a protective immune response against influenza virus by administering to the individual an immunologically effective amount of any of the above polypeptides in a physiologically acceptable carrier.

Additionally, the invention includes recombinant influenza virus that comprises one or more of the polypeptides or polynucleotides above, in addition to immunogenic compositions comprising an immunologically effective amount of such recombinant influenza virus. Methods for stimulating the immune system of an individual to produce a protective immune response against influenza virus, through administering an immunologically effective amount of such recombinant influenza virus in a physiologically acceptable carrier are also part of the invention.

In other aspects, the invention comprises an isolated or recombinant nucleic acid that is selected from: any one of the polynucleotide sequences SEQ ID NO:1 through SEQ ID NO:10 or SEQ ID NO:21 through SEQ ID NO:26 or SEQ ID NO:33 through SEQ ID NO:38, or SEQ ID NO:45 (or complementary sequences thereof), any one of the polynucleotide sequences encoding a polypeptide of SEQ ID NO:11 through SEQ ID NO:20 or SEQ ID NO:27 through SEQ ID NO:32 or SEQ ID NO:39 through SEQ ID NO:44 (or complementary polynucleotide sequences thereof), a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of any of the above polynucleotide sequences, and a polynucleotide sequence comprising all or a fragment of any of such polynucleotide sequences wherein the sequence preferably encodes a hemagglutinin or neuraminidase polypeptide or a fragment of a hemagglutinin or neuraminidase polypeptide. The invention also includes an isolated or recombinant nucleic acid that encodes an amino acid sequence which is substantially identical over at least about 300 amino acids of any polypeptide encoded by the above nucleic acids, or over at least about 350 amino acids; over at least about 400 amino acids; over at least about 450 amino acids; over at least about 500 amino acids; over at least about 502 amino acids; over at least about 550 amino acids; over at least about 559 amino acids; over at least about 565 amino acids; or over at least about 566 amino acids of any polypeptide encoded by the above nucleic acids. Again, in situations wherein the amino acid is less than, e.g., 566, 565, 559, etc. in length (e.g., see, "SEQUENCES") then it should be understood that the length is optionally less than 566, 565, 559, etc. The invention also includes any of the above nucleic acids that comprise a polynucleotide encoding a hemagglutinin or neuraminidase polypeptide, or one or more fragments of one or more hemagglutinin or neuraminidase polypeptide. Other aspects of the invention include isolated or recombinant nucleic acids that encode a polypeptide (optionally a hemagglutinin or neuraminidase polypeptide) whose sequence has at least 98% identity, at least 98.5% identity, at least 99% identity, at least 99.2% identity, at least 99.4% identity, at least 99.6% identity, at least 99.8% identity, or at least 99.9% identity to at least one of the above described polynucleotides. The invention also includes isolated or recombinant nucleic acids encoding a polypeptide of hemagglutinin or neuraminidase produced by mutating or recombining one or more above described polynucleotide sequences. The polynucleotide sequences of the invention can optionally comprise one or more of, e.g., a leader sequence, a precursor sequence, or an epitope tag sequence or the like, and can optionally encode a fusion protein (e.g., with one or more additional nucleic acid sequences).

In yet other embodiments, the invention comprises a composition of matter having two or more above described nucleic acids (e.g., a library comprising at least about 2, 5, 10, 50 or more nucleic acids). Such compositions can optionally be produced by cleaving one or more above described nucleic acid (e.g., mechanically, chemically, enzymatically with a restriction endonuclease/RNAse/DNAse, etc.). Other compositions of the invention include, e.g., compositions produced by incubating one or more above described nucleic acid in the presence of deoxyribonucleotide triphosphates and a thermostable nucleic acid polymerase.

The invention also encompasses cells comprising at least one of the above described nucleic acids, or a cleaved or amplified fragment or product thereof. Such cells can optionally express a polypeptide encoded by such nucleic acid. Other embodiments of the invention include vectors (e.g., plasmids, cosmids, phage, viruses, virus fragments, etc.) comprising any of above described nucleic acids. Such vectors can optionally comprise an expression vector. Preferred expression vectors of the invention include, but are not limited to, vectors comprising pol I promoter and terminator sequences or vectors using both the pol I and pol II promoters "the polI/polII promoter system" (e.g., Zobel et al., Nucl. Acids Res. 1993, 21:3607; US20020164770; Neumann et al., Proc. Natl. Acad. Sci. USA 1999, 96:9345; Fodor et al., J. Virol. 1999, 73:9679; and US20030035814). Cells transduced by such vectors are also within the current invention.

In some embodiments, the invention encompasses a virus (e.g., an influenza virus) comprising one or more above described nucleic acids (e.g., encoding hemagglutinin and/or neuraminidase), or one or more fragments thereof. Immunogenic compositions comprising such virus are also part of the current invention. Such viruses can comprises a reassortment virus such as a 6:2 reassortment virus (e.g., comprising 6 genes encoding regions from one or more donor virus and 2 genes encoding regions from one or more above described nucleotide sequence (or one or more fragment thereof) which can optionally comprise hemagglutinin and/or neuraminidase). Reassortment viruses (optionally live viruses) of the invention can include donor viruses that are one or more of, e.g., cold-sensitive, cold-adapted, or an attenuated. For example, reassortment viruses can comprise e.g., A/Ann Arbor/6/60, PR8, etc. Reassortment viruses of the invention may alternatively exclude A/Ann Arbor/6/60. One preferred embodiment of the invention is a reassortant influenza virus, wherein the virus is a 6:2 reassortment influenza virus and comprises 6 gene encoding regions from A/Ann Arbor/6/60 and 2 gene encoding regions that encode a polypeptide selected from the group consisting of: the polypeptides of SEQ ID NOS:11-20, SEQ ID NOS:27-32, and SEQ ID NOS: 39-44. In an alternative embodiment, a reassortant influenza virus of the invention includes a 6:2 reassortment influenza virus, wherein said virus comprises 6 gene encoding regions from one or more donor viruses other than A/Ann Arbor/6/60 and 2 gene encoding regions that encode a polypeptide selected from the group consisting of: the polypeptides of SEQ ID NOS:11-20, SEQ ID NOS:27-32, and SEQ ID NOS:

39-44. In another alternative embodiment, a reassortant influenza virus of the invention includes a 6:2 reassortment influenza virus, wherein said virus comprises 6 gene encoding regions from one or more donor viruses other than A/Ann Arbor/6/60 and 2 gene encoding regions, wherein the 2 gene encoding regions are HA or NA polypeptides from any pandemic influenza strain. Methods of producing recombinant influenza virus through culturing a host cell harboring an influenza virus in a suitable culture medium under conditions permitting expression of nucleic acid and, isolating the recombinant influenza virus from one or more of the host cell or the medium are also part of the invention.

In other embodiments herein, the invention comprises immunogenic compositions having an immunologically effective amount of any of the above described recombinant influenza virus. Other embodiments include methods for stimulating the immune system of an individual to produce a protective immune response against influenza virus by administering to the individual an immunologically effective amount of any of the recombinant influenza virus described above (optionally in a physiologically effective carrier).

Other aspects of the invention include methods of producing an isolated or recombinant polypeptide by culturing any host cell above, in a suitable culture medium under conditions permitting expression of nucleic acid and, isolating the polypeptide from one or more of the host cells or the medium in which is the cells are grown.

Immunogenic compositions are also features of the invention. For example, immunogenic compositions comprising one or more of any of the polypeptides and/or nucleic acids described above and, optionally, an excipient such as a pharmaceutically acceptable excipient or one or more pharmaceutically acceptable administration component. Immunogenic compositions of the invention can also comprise any one or more above described virus as well (e.g., along with one or more pharmaceutically acceptable administration component).

Methods of producing immunogenic responses in a subject through administration of an effective amount of any of the above viruses (or immunogenic compositions) to a subject are also within the current invention. Additionally, methods of prophylactic or therapeutic treatment of a viral infection (e.g., viral influenza) in a subject through administration of any one or more above described virus (or immunogenic compositions) in an amount effective to produce an immunogenic response against the viral infection are also part of the current invention. Subjects for such treatment can include mammals (e.g., humans). Such methods can also comprise in vivo administration to the subject as well as in vitro or ex vivo administration to one or more cells of the subject. Additionally, such methods can also comprise administration of a composition of the virus and a pharmaceutically acceptable excipient that are administered to the subject in an amount effect to prophylactically or therapeutically treat the viral infection.

In other aspects the invention includes compositions of matter comprising nucleic acid sequences encoding hemagglutinin and/or neuraminidase polypeptides of one or more pandemic influenza strain and nucleic acid sequences encoding one or more polypeptide of A/Ann Arbor/6/60. Additionally, the invention includes compositions of matter comprising nucleic acid sequences encoding hemagglutinin and/or neuraminidase polypeptides of one or more pandemic influenza strain and nucleic acid sequences encoding one or more polypeptide of PR8 or A/Ann Arbor/6/60. Such sequences can include those listed in the "SEQUENCES" herein. Additionally, preferred embodiments of the invention include compositions of matter comprising sequences encoding hemagglutinin and/or neuraminidase of one or more pandemic influenza strain and nucleic acid sequences encoding a selected backbone strain in a 6:2 reassortment. Such compositions preferably include sequences encoding the hemagglutinin and neuraminidase selected from the "SEQUENCES" herein and a backbone strain, wherein the backbone strain is PR8 or A/Ann Arbor/6/60. The invention also includes such compositions as described above wherein the hemagglutinin comprises a modified polybasic cleavage site. The invention also includes live attenuated influenza vaccine comprising such above compositions.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and appendix.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**: Shows modifications engineered into the HA gene of VN/1203/2004 to remove the polybasic cleavage site. FIG. **1** discloses SEQ ID NOS 46-47 and 55-56, respectively, in order of appearance.

FIG. **2**: Displays results showing that intranasally administered H5N1 ca reassortant viruses do not replicate in chickens.

FIG. **3**: Illustrates that the H5N1/AA ca vaccine candidates are not lethal to mice.

FIG. **4**: Illustrates that the 1997 and 2004 H5N1 ca reassortant viruses are restricted in replication in mice.

FIG. **5**: Illustrates that the reassortant H5N1/AA ca influenza viruses are restricted in replication in lungs of mice.

FIG. **6**: Shows the serum HAI Ab titers elicited in mice following a single i.n. dose of vaccine.

FIG. **7**: Shows serum neutralizing Ab titers elicited in mice following a single i.n. dose of vaccine.

FIG. **8**: Illustrates that H5N1 ca reassortant viruses protect mice from lethal challenges with 50, 500 or 5000 LD_{50} of wild-type H5N1 viruses.

FIG. **9**: Illustrates the efficacy of protection from pulmonary replication of homologous and heterologous H5N1 challenge viruses in mice.

FIG. **10**: Illustrates the efficacy of protection from replication of homologous and heterologous H5N1 challenge viruses in the upper respiratory tract of mice.

FIG. **11**: Illustrates the efficacy of protection conferred by 2004 H5N1 ca vaccine against high dose (10_5TCID$_{50}$) challenge with homologous or heterologous H5N1 wt viruses in mice.

FIG. **12**: Illustrates the efficacy of protection conferred by 1997 and 2003 H5N1 ca vaccines against high dose (10_5TCID$_{50}$) challenges with homologous or heterologous H5N1 wild-type viruses in mice.

FIG. **13**: Illustrates the efficacy of protection conferred by 2004 H5N1 ca vaccine against low or high doses of homologous H5N1 wild-type virus challenges in mice.

FIG. **14**: Shows modifications that can be engineered into the HA gene of A/Netherland/219/03 HA to remove the polybasic cleavage site. FIG. **14** discloses SEQ ID NOS 57-58 and 48-53, respectively, in order of appearance.

FIG. **15**: H5N1 ca vaccines elicit serum neutralizing antibody titers in mice. Sera were collected before (prebleed) and 28 days following each dose of vaccine; an undetectable titer is assigned a value of 10.

FIG. **16**: H6 ca viruses are attenuated in ferrets. *EID_{50}/g for lungs; PFU/g for nasal turbinates. 10^7 $TCID_{50}$ inoculated intranasally, tissues were harvested on day 3 post-infection.

FIG. **17**: Immunogenicity of H6 ca vaccines in ferrets.

FIGS. **18**(*a*) and **18**(*b*): Efficacy of H6 ca vaccines in ferrets. Virus titer was measured in (a) lungs and (b) nasal turbinates. Vaccine: 1 dose of $7\log_{10}$ PFU. Challenge: $7\log_{10}$ PFU; 3 days post-challenge.

FIG. **19**: H7N3 BC 04 ca is attenuated in ferrets. Inoculum: 10^7 $TCID_{50}$ in 0.5 mL intranasally. Tissues were harvested on day 3 post-infection.

FIG. **20**: H7N3 BC 2004 ca is immunogenic in mice. a: Reciprocal geometric mean of serum neutralizing antibody titers against ck/BC/CN-6/04 wt. b: $p<0.05$ (Mann-Whitney U-test) compared to neutralization titers at 28 days post-infection.

FIG. **21**(*a*)-**21**(*i*): H7N3 BC 04 ca is efficacious against lethal challenge with H7 viruses in mice. Efficacy against a lethal challenge of 50 LD_{50} A/ck/BC/CN-7/04: four weeks following immunization with a single dose (a), eight weeks following immunization with a single dose (b), or eight weeks following immunization with 2 doses (2 doses administered at 4 weeks apart) (c). Efficacy against a lethal challenge of 50 LD_{50} A/NL/219/03: four weeks following immunization with a single dose (d), eight weeks following immunization with a single dose (e), or eight weeks following immunization with 2 doses (2 doses administered at 4 weeks apart) (f). Efficacy against a lethal challenge of 50 LD_{50} A/tk/Eng/63: four weeks following immunization with a single dose (g), eight weeks following immunization with a single dose (h), or eight weeks following immunization with 2 doses (2 doses administered at 4 weeks apart) (i).

FIG. **22**(*a*) and (*b*): H7N3 BC 04 ca vaccine is efficacious in mice. Virus titer was measured at 8 weeks in (a) nasal turbinates and (b) lungs.

FIG. **23**(*a*) and (*b*): H9N2 G9/AA ca is attenuated in ferrets. Virus titer was measured in (a) nasal turbinates and (b) lungs.

FIG. **24**(*a*) and (*b*): Efficacy of the H9N2 ca vaccine in mice.

FIG. **25**: Replication of H9N2 G9/AA ca is highly restricted in healthy adults.

FIG. **26**: HI antibody responses to $10^{7.0}$ $TCID_{50}$ of H9N2 G9/AA ca in healthy adults.

FIG. **27**: Replication of H5N1 VN2004 A/AA ca is highly restricted in healthy adults.

FIG. **28**: HI antibody responses to $10^{6.7}$ $TCID_{50}$ of VN2004 A/AA ca in healthy adults.

DETAILED DESCRIPTION

The present invention includes polypeptide and polynucleotide sequences of influenza hemagglutinin and neuraminidase as well as vectors, compositions and the like comprising such sequences and methods of their use. Additional features of the invention are described in more detail herein.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. The following definitions supplement those in the art and are directed to the current application and are not necessarily to be imputed to any related or unrelated case, e.g., to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a virus" includes a plurality of viruses; reference to a "host cell" includes mixtures of host cells, and the like.

An "amino acid sequence" is a polymer of amino acid residues (a protein, polypeptide, etc.) or a character string representing an amino acid polymer, depending on context.

The terms "nucleic acid," "polynucleotide," "polynucleotide sequence" and "nucleic acid sequence" refer to single-stranded or double-stranded deoxyribonucleotide or ribonucleotide polymers, chimeras or analogues thereof, or a character string representing such, depending on context. As used herein, the term optionally includes polymers of analogs of naturally occurring nucleotides having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (e.g., peptide nucleic acids). Unless otherwise indicated, a particular nucleic acid sequence of this invention optionally encompasses complementary sequences in addition to the sequence explicitly indicated. From any specified polynucleotide sequence, either the given nucleic acid or the complementary polynucleotide sequence (e.g., the complementary nucleic acid) can be determined.

The term "nucleic acid" or "polynucleotide" also encompasses any physical string of monomer units that can be corresponded to a string of nucleotides, including a polymer of nucleotides (e.g., a typical DNA or RNA polymer), PNAs, modified oligonucleotides (e.g., oligonucleotides comprising bases that are not typical to biological RNA or DNA in solution, such as 2'-O-methylated oligonucleotides), and the like. A nucleic acid can be e.g., single-stranded or double-stranded.

A "subsequence" is any portion of an entire sequence, up to and including the complete sequence. Typically, a subsequence comprises less than the full-length sequence. A "unique subsequence" is a subsequence that is not found in any previously determined influenza polynucleotide or polypeptide sequence.

The term "variant" with respect to a polypeptide refers to an amino acid sequence that is altered by one or more amino acids with respect to a reference sequence. The variant can have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. Alternatively, a variant can have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variation can also include amino acid deletion or insertion, or both. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without eliminating biological or immunological activity can be found using computer programs well known in the art, for example, DNASTAR software. Examples of conservative substitutions are also described herein.

The term "gene" is used broadly to refer to any nucleic acid associated with a biological function. Thus, genes include coding sequences and/or the regulatory sequences required for their expression. The term "gene" applies to a specific genomic sequence, as well as to a cDNA or an mRNA encoded by that genomic sequence.

Genes also include non-expressed nucleic acid segments that, for example, form recognition sequences for other proteins. Non-expressed regulatory sequences include "promoters" and "enhancers," to which regulatory proteins such as transcription factors bind, resulting in transcription of adja-

cent or nearby sequences. A "tissue specific" promoter or enhancer is one that regulates transcription in a specific tissue type or cell type, or types.

"Expression of a gene" or "expression of a nucleic acid" means transcription of DNA into RNA (optionally including modification of the RNA, e.g., splicing), translation of RNA into a polypeptide (possibly including subsequent modification of the polypeptide, e.g., post-translational modification), or both transcription and translation, as indicated by the context.

An "open reading frame" or "ORF" is a possible translational reading frame of DNA or RNA (e.g., of a gene), which is capable of being translated into a polypeptide. That is, the reading frame is not interrupted by stop codons. However, it should be noted that the term ORF does not necessarily indicate that the polynucleotide is, in fact, translated into a polypeptide.

The term "vector" refers to the means by which a nucleic acid can be propagated and/or transferred between organisms, cells, or cellular components. Vectors include plasmids, viruses, bacteriophages, pro-viruses, phagemids, transposons, artificial chromosomes, and the like, that replicate autonomously or can integrate into a chromosome of a host cell. A vector can also be a naked RNA polynucleotide, a naked DNA polynucleotide, a polynucleotide composed of both DNA and RNA within the same strand, a poly-lysine-conjugated DNA or RNA, a peptide-conjugated DNA or RNA, a liposome-conjugated DNA, or the like, that is not autonomously replicating. In many, but not all, common embodiments, the vectors of the present invention are plasmids.

An "expression vector" is a vector, such as a plasmid that is capable of promoting expression, as well as replication of a nucleic acid incorporated therein. Typically, the nucleic acid to be expressed is "operably linked" to a promoter and/or enhancer, and is subject to transcription regulatory control by the promoter and/or enhancer.

A "bi-directional expression vector" is characterized by two alternative promoters oriented in the opposite direction relative to a nucleic acid situated between the two promoters, such that expression can be initiated in both orientations resulting in, e.g., transcription of both plus (+) or sense strand, and negative (−) or antisense strand RNAs.

A "polypeptide" is a polymer comprising two or more amino acid residues (e.g., a peptide or a protein). The polymer can optionally comprise modifications such as glycosylation or the like. The amino acid residues of the polypeptide can be natural or non-natural and can be unsubstituted, unmodified, substituted or modified.

In the context of the invention, the term "isolated" refers to a biological material, such as a virus, a nucleic acid or a protein, which is substantially free from components that normally accompany or interact with it in its naturally occurring environment. The isolated biological material optionally comprises additional material not found with the biological material in its natural environment, e.g., a cell or wild-type virus. For example, if the material is in its natural environment, such as a cell, the material can have been placed at a location in the cell (e.g., genome or genetic element) not native to such material found in that environment. For example, a naturally occurring nucleic acid (e.g., a coding sequence, a promoter, an enhancer, etc.) becomes isolated if it is introduced by non-naturally occurring means to a locus of the genome (e.g., a vector, such as a plasmid or virus vector, or amplicon) not native to that nucleic acid. Such nucleic acids are also referred to as "heterologous" nucleic acids. An isolated virus, for example, is in an environment (e.g., a cell culture system, or purified from cell culture) other than the native environment of wild-type virus (e.g., the nasopharynx of an infected individual).

The term "chimeric" or "chimera," when referring to a virus, indicates that the virus includes genetic and/or polypeptide components derived from more than one parental viral strain or source. Similarly, the term "chimeric" or "chimera," when referring to a viral protein, indicates that the protein includes polypeptide components (i.e., amino acid subsequences) derived from more than one parental viral strain or source.

The term "recombinant" indicates that the material (e.g., a nucleic acid or protein) has been artificially or synthetically (non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. Specifically, e.g., an influenza virus is recombinant when it is produced by the expression of a recombinant nucleic acid. For example, a "recombinant nucleic acid" is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling or other procedures, or by chemical or other mutagenesis; a "recombinant polypeptide" or "recombinant protein" is a polypeptide or protein which is produced by expression of a recombinant nucleic acid; and a "recombinant virus", e.g., a recombinant influenza virus, is produced by the expression of a recombinant nucleic acid.

The term "reassortant," when referring to a virus, indicates that the virus includes genetic and/or polypeptide components derived from more than one parental viral strain or source. For example, a 7:1 reassortant includes 7 viral genomic segments (or gene segments) derived from a first parental virus, and a single complementary viral genomic segment, e.g., encoding a hemagglutinin or neuraminidase of the invention. A 6:2 reassortant includes 6 genomic segments, most commonly the 6 internal genes from a first parental virus, and two complementary segments, e.g., hemagglutinin and neuraminidase, from a different parental virus.

The term "introduced" when referring to a heterologous or isolated nucleic acid refers to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid can be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA). The term includes such methods as "infection," "transfection," "transformation" and "transduction." In the context of the invention a variety of methods can be employed to introduce nucleic acids into cells, including electroporation, calcium phosphate precipitation, lipid mediated transfection (lipofection), etc.

The term "host cell" means a cell that contains a heterologous nucleic acid, such as a vector, and supports the replication and/or expression of the nucleic acid. Host cells can be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, avian or mammalian cells, including human cells. Exemplary host cells can include, e.g., Vero (African green monkey kidney) cells, BHK (baby hamster kidney) cells, primary chick kidney (PCK) cells, Madin-Darby Canine Kidney (MDCK) cells, Madin-Darby Bovine Kidney (MDBK) cells, 293 cells (e.g., 293T cells), and COS cells (e.g., COS1, COS7 cells), etc.

An "immunologically effective amount" of influenza virus is an amount sufficient to enhance an individual's (e.g., a human's) own immune response against a subsequent exposure to influenza virus. Levels of induced immunity can be monitored, e.g., by measuring amounts of neutralizing secre-

tory and/or serum antibodies, e.g., by plaque neutralization, complement fixation, enzyme-linked immunosorbent, or microneutralization assay.

A "protective immune response" against influenza virus refers to an immune response exhibited by an individual (e.g., a human) that is protective against disease when the individual is subsequently exposed to and/or infected with such influenza virus. In some instances, the influenza virus (e.g., naturally circulating) can still cause infection, but it cannot cause a serious infection. Typically, the protective immune response results in detectable levels of host engendered serum and secretory antibodies that are capable of neutralizing virus of the same strain and/or subgroup (and possibly also of a different, non-vaccine strain and/or subgroup) in vitro and in vivo.

As used herein, an "antibody" is a protein comprising one or more polypeptides substantially or partially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A typical immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chains respectively. Antibodies exist as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab')2 dimer into a Fab' monomer. The Fab' monomer is essentially a Fab with part of the hinge region (see, Fundamental Immunology, W. E. Paul, ed., Raven Press, N.Y. (1999), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein, includes antibodies or fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies. Antibodies include, e.g., polyclonal antibodies, monoclonal antibodies, multiple or single chain antibodies, including single chain Fv (sFv or scFv) antibodies in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide, and humanized or chimeric antibodies.

Influenza Virus

The polypeptides and polynucleotides of the invention, e.g., SEQ ID NO: 1-45, are variants of influenza HA and NA sequences. In general, influenza viruses are made up of an internal ribonucleoprotein core containing a segmented single-stranded RNA genome and an outer lipoprotein envelope lined by a matrix protein. The genome of influenza viruses is composed of eight segments of linear (−) strand ribonucleic acid (RNA), encoding the immunogenic hemagglutinin (HA) and neuraminidase (NA) proteins, and six internal core polypeptides: the nucleocapsid nucleoprotein (NP); matrix proteins (M); non-structural proteins (NS); and 3 RNA polymerase (PA, PB1, PB2) proteins. During replication, the genomic viral RNA is transcribed into (+) strand messenger RNA and (−) strand genomic cRNA in the nucleus of the host cell. Each of the eight genomic segments is packaged into ribonucleoprotein complexes that contain, in addition to the RNA, NP and a polymerase complex (PB1, PB2, and PA).

Influenza is commonly grouped into influenza A and influenza B categories. Influenza A and influenza B viruses each contain eight segments of single stranded RNA with negative polarity. The influenza A genome encodes eleven polypeptides. Segments 1-3 encode three polypeptides, making up a RNA-dependent RNA polymerase. Segment 1 encodes the polymerase complex protein PB2. The remaining polymerase proteins PB1 and PA are encoded by segment 2 and segment 3, respectively. In addition, segment 1 of some influenza strains encodes a small protein, PB1-F2, produced from an alternative reading frame within the PB1 coding region. Segment 4 encodes the hemagglutinin (HA) surface glycoprotein involved in cell attachment and entry during infection. Segment 5 encodes the nucleocapsid nucleoprotein (NP) polypeptide, the major structural component associated with viral RNA. Segment 6 encodes a neuraminidase (NA) envelope glycoprotein. Segment 7 encodes two matrix proteins, designated M1 and M2, which are translated from differentially spliced mRNAs. Segment 8 encodes NS1 and NS2, two nonstructural proteins, which are translated from alternatively spliced mRNA variants. The eight genome segments of influenza B encode 11 proteins. The three largest genes code for components of the RNA polymerase, PB1, PB2 and PA. Segment 4 encodes the HA protein. Segment 5 encodes NP. Segment 6 encodes the NA protein and the NB protein. Both proteins, NB and NA, are translated from overlapping reading frames of a bicistronic mRNA. Segment 7 of influenza B also encodes two proteins: M1 and BM2. The smallest segment encodes two products: NS1 is translated from the full length RNA, while NS2 is translated from a spliced mRNA variant.

Influenza Virus Vaccines

The sequences, compositions and methods herein are primarily, but not solely, concerned with production of influenza viruses for vaccines. Historically, influenza virus vaccines have primarily been produced in embryonated hen eggs using strains of virus selected or based on empirical predictions of relevant strains. More recently, reassortant viruses have been produced that incorporate selected hemagglutinin and/or neuraminidase antigens in the context of an approved attenuated, temperature sensitive master strain. Following culture of the virus through multiple passages in hen eggs, influenza viruses are recovered and, optionally, inactivated, e.g., using formaldehyde and/or β-propiolactone (or alternatively used in live attenuated vaccines). Thus, it will be appreciated that HA and NA sequences (e.g., SEQ ID NO: 1-45) are quite useful in constructing influenza vaccines. The current invention includes viruses/vaccines comprising HA and/or NA sequences of pandemic influenza strains (including wherein the HA sequences comprise modified polybasic cleavage sites such as the modifications described herein); and including wherein the viruses/vaccines comprise a ca backbone such as A/AA/6/60 or the backbone of PR8.

Attempts at producing recombinant and reassortant vaccines in cell culture have been hampered by the inability of some of the strains approved for vaccine production to grow efficiently under standard cell culture conditions. However, prior work by the inventors and their coworkers provided a vector system, and methods for producing recombinant and

reassortant viruses in culture, thus, making it possible to rapidly produce vaccines corresponding to one or many selected antigenic strains of virus, e.g., either A or B strains, various subtypes or substrains, etc., e.g., comprising the HA and/or NA sequences herein. See, Multi-Plasmid System for the production of Influenza virus, U.S. Application No. 60/420,708, filed Oct. 23, 2002, U.S. application Ser. No. 10/423,828, filed Apr. 25, 2003 and U.S. Application 60/574, 117 filed May 24, 2004. Typically, the cultures are maintained in a system, such as a cell culture incubator, under controlled humidity and CO_2, at constant temperature using a temperature regulator, such as a thermostat to insure that the temperature does not exceed 35° C. Reassortant influenza viruses can be readily obtained by introducing a subset of vectors corresponding to genomic segments of a master influenza virus, in combination with complementary segments derived from strains of interest (e.g., HA and/or NA antigenic variants herein). Typically, the master strains are selected on the basis of desirable properties relevant to vaccine administration. For example, for vaccine production, e.g., for production of a live attenuated vaccine, the master donor virus strain may be selected for an attenuated phenotype, cold adaptation and/or temperature sensitivity. As explained elsewhere herein and, e.g., in U.S. patent application Ser. No. 10/423,828, etc., various embodiments of the invention utilize A/Ann Arbor (AA)/6/60 influenza strain as a "backbone" upon which to add HA and/or NA genes (e.g., such as those sequences listed herein, etc.) to create desired reassortant viruses. Thus, for example, in a 6:2 reassortant, 2 genes (i.e., NA and HA) would be from the influenza strain(s) against which an immunogenic reaction is desired, while the other 6 genes would be from the Ann Arbor strain, or other backbone strain, etc. The Ann Arbor virus is useful for its cold adapted, attenuated, temperature sensitive attributes. Of course, it will be appreciated that the HA and NA sequences herein are capable of reassortment with a number of other virus genes or virus types (e.g., a number of different "backbones" such as PR8, etc., containing the other influenza genes present in a reassortant, namely, the non-HA and non-NA genes

Various embodiments herein can comprise live attenuated vaccines, having the HA and/or NA sequences herein, for pandemic influenza. Such vaccines typically comprise, e.g., the HA and/or NA sequences of SEQ ID NO: 11-20, 27-32, or 39-44, or their corresponding encoding nucleotides of SEQ ID NO: 1-10, 21-26, 33-38, or 45. One problem arising from growth of vaccine virus strains (e.g., reassortants) in eggs is that avian strains (which can be involved in pandemics) can kill the eggs in which the vaccines are to be produced and are, thus, hard to manipulate, produce, etc. through use of traditional (non-plasmid rescue) reassortant production. Such avian strains are of interest since evidence indicates they can result in influenza in humans and possible pandemics. Thus, use of plasmid-rescue systems to create/manipulate influenza reassortants with pandemic strains such as various avian sequences (e.g., the HA and NA sequences herein) are quite desirable and are features of the invention. It will be appreciated, however, that the current sequences are also capable of use with non-plasmid or traditional systems.

Aquatic birds (among others) can be infected by influenza A viruses of 15 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes. Such birds can serve as a reservoir from which novel influenza subtypes can be introduced into human populations and cause pandemics. The observation that avian H7N7 influenza A viruses infected humans in The Netherlands in 2003 and avian H5N1 and H9N2 viruses infected humans in Hong Kong and China earlier, raise concerns that these (and other) subtypes have the potential to cause pandemics. Thus, vaccines are needed to prevent human infections with avian influenza A viruses. Live, attenuated influenza A virus vaccines against human influenza viruses were recently licensed in the United States. See above. Such vaccines are reassortant H1N1 and H3N2 viruses in which the internal protein genes of A/Ann Arbor (AA)/6/60 (H2N2) cold adapted (ca) virus confer the cold adapted, attenuation and temperature sensitive phenotypes of the AA ca virus on the reassortant viruses (i.e., the ones having the hemagglutinin and neuraminidase genes from the non-Ann Arbor strain). Classical genetic reassortment and plasmid-based reverse genetics techniques have been applied to generate reassortant viruses that contain the hemagglutinin and neuraminidase genes from avian influenza A viruses (H4-H14 subtypes) and six internal gene segments from the AA ca virus. Such reassortant viruses are features of the invention. See the HA and NA gene sequences below. These viruses bear biological properties that are desirable in candidate vaccines because the phenotypes associated with the AA ca virus are present in the reassortant viruses. The generation and evaluation of these reassortant viruses as seed viruses for vaccines are important steps in pandemic preparedness. It is contemplated that clinical trials can establish the safety, infectivity and immunogenicity of such live attenuated pandemic vaccines. Other embodiments of the invention include reassortant viruses (e.g., those used in vaccines) comprising pandemic antigenic genes HA and/or NA from, e.g., avian, porcine, etc., pandemic virus strains in addition to those listed herein, to produce pandemic vaccines which are created through plasmid-rescue reassortment (e.g., reassortment with A/Ann Arbor 6/60 (i.e., A/AA/6/60), PR8, etc. Methods of construction and use of such viruses and vaccines are also included. "Pandemic virus strains" as used herein is defined as an influenza strain A virus subtype that it is not circulating in the human population, that is declared to be a pandemic strain by the Centers for Disease Control or the World Health Organization or generally acknowledged as such within the scientific community.

In various embodiments herein, the antigenic sequences (e.g., the HA sequences) as well as viruses and vaccines from such viruses comprise modified polybasic cleavage sites. Some highly pathogenic avian pandemic influenza strains comprise multiple basic amino acid cleavage sites within hemagglutinin sequences. See, e.g., Li et al., *J. of Infectious Diseases,* 179:1132-8, 1999. Such cleavage sites, in typical embodiments herein, are, e.g., modified or altered in their sequences in comparison to the wild-type sequences from which the current sequences are derived (e.g., to disable the cleavage or reduce the cleavage there, etc.). Such modifications/alterations can be different in different strains due to the various sequences of the cleavage sites in the wild-type sequences. For example, 4 polybasic residues (RRKK) (SEQ ID No: 54) at 326-329 of mature H5 are typically removed in sequences herein (as compared to wt). See "SEQUENCES." In various embodiments, the polybasic cleavage sites can be modified in a number of ways (all of which are contained within the invention). For example, the polybasic cleavage site can be removed one amino acid at a time (e.g., one R removed, two R5 removed, RRK removed, or RRKK (SEQ ID NO: 54) removed). Additionally, the amino acid residue directly upstream of the cleavage site can also be removed or altered (e.g., from an R to a T, etc.); also, the nucleotides encoding the amino acid residue directly after the cleavage site can also be modified. See, e.g., FIG. **1** for an illustration of cleavage site modification. In addition, hemagglutinin polypeptide sequences of influenza virus comprise amino terminal signal peptide sequences, thus, the hemagglutinin polypeptide sequences of the invention include both the

mature (amino terminal signal peptide cleaved) form of the hemagglutinin polypeptides and the pre-cleaved form of hemagglutinin. The cleavage sites of any hemagglutinin polypeptide sequence of any influenza strain can be routinely measured or predicted using any number of methods in the art.

The terms "temperature sensitive," "cold adapted" and "attenuated" as applied to viruses (typically used as vaccines or for vaccine production) which optionally encompass the current sequences, are well known in the art. For example, the term "temperature sensitive" (ts) indicates, e.g., that the virus exhibits a 100 fold or greater reduction in titer at 39° C. relative to 33° C. for influenza A strains, or that the virus exhibits a 100 fold or greater reduction in titer at 37° C. relative to 33° C. for influenza B strains. The term "cold adapted" (ca) indicates that the virus exhibits growth at 25° C. within 100 fold of its growth at 33° C., while the term "attenuated" (att) indicates that the virus replicates in the upper airways of ferrets but is not detectable in their lung tissues, and does not cause influenza-like illness in the animal. It will be understood that viruses with intermediate phenotypes, i.e., viruses exhibiting titer reductions less than 100 fold at 39° C. (for A strain viruses) or 37° C. (for B strain viruses), or exhibiting growth at 25° C. that is more than 100 fold than its growth at 33° C. (e.g., within 200 fold, 500 fold, 1000 fold, 10,000 fold less), and/or exhibit reduced growth in the lungs relative to growth in the upper airways of ferrets (i.e., partially attenuated) and/or reduced influenza like illness in the animal, are also useful viruses and can be used in conjunction with the HA and NA sequences herein.

Again, the HA and NA sequences of the current invention are optionally utilized in the production of or in reassortment vaccines (and/or in other ts, cs, ca, and/or att viruses and vaccines). However, it should be noted that the HA and NA sequences, etc. of the invention are not limited to specific vaccine compositions or production methods, and can, thus, be utilized in substantially any vaccine type or vaccine production method which utilizes strain specific HA and NA antigens (e.g., any of SEQ ID NO: 11-20, 27-32, or 39-44 or the corresponding nucleotides encoding the specific HA and NA antigens, e.g., SEQ ID NO: 1-10, 21-26, 33-38, or 45).

FluMist™

As mentioned previously, numerous examples and types of influenza vaccine exist. An exemplary influenza vaccine is FluMist™ which is a live, attenuated vaccine that protects children and adults from influenza illness (Belshe et al. (1998) *The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine in children N Engl J Med* 338:1405-12; Nichol et al. (1999) *Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial JAMA* 282: 137-44). In typical, and preferred, embodiments, the methods and compositions of the current invention are preferably adapted to/used with production of FluMist™ vaccine. However, it will be appreciated by those skilled in the art that the sequences, methods, compositions, etc. herein are also adaptable to production of similar or even different viral vaccines.

FluMist™ vaccine strains contain, e.g., HA and NA gene segments derived from the strains (e.g., wild-type strains) to which the vaccine is addressed along with six gene segments, PB1, PB2, PA, NP, M and NS, from a common master donor virus (MDV). The HA sequences herein, thus, are part of various FluMist™ formulations. The MDV for influenza A strains of FluMist™ (MDV-A), was created by serial passage of the wild-type A/Ann Arbor/6/60 (A/AA/6/60) strain in primary chicken kidney tissue culture at successively lower temperatures (Maassab (1967) *Adaptation and growth characteristics of influenza virus at* 25 *degrees C. Nature* 213:612-4). MDV-A replicates efficiently at 25° C. (ca, cold adapted), but its growth is restricted at 38 and 39° C. (ts, temperature sensitive). Additionally, this virus does not replicate in the lungs of infected ferrets (att, attenuation). The ts phenotype is believed to contribute to the attenuation of the vaccine in humans by restricting its replication in all but the coolest regions of the respiratory tract. The stability of this property has been demonstrated in animal models and clinical studies. In contrast to the ts phenotype of influenza strains created by chemical mutagenesis, the ts property of MDV-A does not revert following passage through infected hamsters or in shed isolates from children (for a recent review, see Murphy & Coelingh (2002) *Principles underlying the development and use of live attenuated cold-adapted influenza A and B virus vaccines Viral Immunol* 15:295-323).

Clinical studies in over 20,000 adults and children involving 12 separate 6:2 reassortant strains have shown that these vaccines are attenuated, safe and efficacious (Belshe et al. (1998) *The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine in children N Engl J Med* 338:1405-12; Boyce et al. (2000) *Safety and immunogenicity of adjuvanted and unadjuvanted subunit influenza vaccines administered intranasally to healthy adults Vaccine* 19:217-26; Edwards et al. (1994) *A randomized controlled trial of cold adapted and inactivated vaccines for the prevention of influenza A disease J Infect Dis* 169:68-76; Nichol et al. (1999) *Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial JAMA* 282:137-44). Reassortants carrying the six internal genes of MDV-A and the two HA and NA gene segments of a wild-type virus (i.e., a 6:2 reassortant) consistently maintain ca, ts and att phenotypes (Maassab et al. (1982) *Evaluation of a cold-recombinant influenza virus vaccine in ferrets J. Infect. Dis.* 146:780-900).

Production of such reassorted virus using B strains of influenza is more difficult, however, recent work (see, e.g., Multi-Plasmid System for the Production of Influenza Virus, U.S. Application No. 60/420,708, filed Oct. 23, 2002, U.S. application Ser. No. 10/423,828, filed Apr. 25, 2003, and U.S. Application No. 60/574,117, filed May 24, 2004) has shown an eight plasmid system for the generation of influenza B virus entirely from cloned cDNA. Methods for the production of attenuated live influenza A and B virus suitable for vaccine formulations, such as live virus vaccine formulations useful for intranasal administration were also shown.

The system and methods described previously are useful for the rapid production in cell culture of recombinant and reassortant influenza A and B viruses, including viruses suitable for use as vaccines, including live attenuated vaccines, such as vaccines suitable for intranasal administration. The sequences (e.g., nucleotide sequences SEQ ID NO: 1-10, 21-26, 33-38, or 45 and the corresponding amino acids encoded by the nucleotide sequences in SEQ ID NO: 11-20, 27-32, or 39-44), methods, etc. of the current invention, are optionally used in conjunction with, or in combination with, such previous work involving, e.g., reassorted influenza viruses for vaccine production to produce viruses for vaccines.

Methods and Compositions for Prophylactic Administration of Vaccines

As stated above, alternatively, or in addition to, use in production of FluMist™ vaccine, the current invention can be used in other vaccine formulations. In general, recombinant and reassortant viruses of the invention (e.g., those comprising polynucleotides of SEQ ID NO:1-10, 21, 23-26, 33-38, or 45 or polypeptides of SEQ ID NO:11-20, 27-32, or 39-44, or

fragments thereof) can be administered prophylactically in an immunologically effective amount and in an appropriate carrier or excipient to stimulate an immune response specific for one or more strains of influenza virus as determined by the HA and/or NA sequence. Typically, the carrier or excipient is a pharmaceutically acceptable carrier or excipient, such as sterile water, aqueous saline solution, aqueous buffered saline solutions, aqueous dextrose solutions, aqueous glycerol solutions, ethanol, or combinations thereof. The preparation of such solutions insuring sterility, pH, isotonicity, and stability is effected according to protocols established in the art. Generally, a carrier or excipient is selected to minimize allergic and other undesirable effects, and to suit the particular route of administration, e.g., subcutaneous, intramuscular, intranasal, etc.

A related aspect of the invention provides methods for stimulating the immune system of an individual to produce a protective immune response against influenza virus. In the methods, an immunologically effective amount of a recombinant influenza virus (e.g., comprising an HA and/or NA molecule of the invention), an immunologically effective amount of a polypeptide of the invention, and/or an immunologically effective amount of a nucleic acid of the invention is administered to the individual in a physiologically acceptable carrier.

Generally, the influenza viruses of the invention are administered in a quantity sufficient to stimulate an immune response specific for one or more strains of influenza virus (i.e., against the HA and/or NA strains of the invention). Preferably, administration of the influenza viruses elicits a protective immune response to such strains. Dosages and methods for eliciting a protective immune response against one or more influenza strains are known to those of skill in the art. See, e.g., U.S. Pat. No. 5,922,326; Wright et al., *Infect. Immun* 37:397-400 (1982); Kim et al., *Pediatrics* 52:56-63 (1973); and Wright et al., *J. Pediatr.* 88:931-936 (1976). For example, influenza viruses are provided in the range of about 1-1000 HID_{50} (human infectious dose), i.e., about 10^5-10^8 pfu (plaque forming units) per dose administered. Typically, the dose will be adjusted within this range based on, e.g., age, physical condition, body weight, sex, diet, time of administration, and other clinical factors. The prophylactic vaccine formulation is systemically administered, e.g., by subcutaneous or intramuscular injection using a needle and syringe, or a needle-less injection device. Alternatively, the vaccine formulation is administered intranasally, either by drops, large particle aerosol (greater than about 10 microns), or spray into the upper respiratory tract. While any of the above routes of delivery results in a protective systemic immune response, intranasal administration confers the added benefit of eliciting mucosal immunity at the site of entry of the influenza virus. For intranasal administration, attenuated live virus vaccines are often preferred, e.g., an attenuated, cold adapted and/or temperature sensitive recombinant or reassortant influenza virus. See above. While stimulation of a protective immune response with a single dose is preferred, additional dosages can be administered, by the same or different route, to achieve the desired prophylactic effect.

Typically, the attenuated recombinant influenza of this invention as used in a vaccine is sufficiently attenuated such that symptoms of infection, or at least symptoms of serious infection, will not occur in most individuals immunized (or otherwise infected) with the attenuated influenza virus. In some instances, the attenuated influenza virus can still be capable of producing symptoms of mild illness (e.g., mild upper respiratory illness) and/or of dissemination to unvaccinated individuals. However, its virulence is sufficiently abrogated such that severe lower respiratory tract infections do not occur in the vaccinated or incidental host.

Alternatively, an immune response can be stimulated by ex vivo or in vivo targeting of dendritic cells with influenza viruses comprising the sequences herein. For example, proliferating dendritic cells are exposed to viruses in a sufficient amount and for a sufficient period of time to permit capture of the influenza antigens by the dendritic cells. The cells are then transferred into a subject to be vaccinated by standard intravenous transplantation methods.

While stimulation of a protective immune response with a single dose is preferred, additional dosages can be administered, by the same or different route, to achieve the desired prophylactic effect. In neonates and infants, for example, multiple administrations may be required to elicit sufficient levels of immunity. Administration can continue at intervals throughout childhood, as necessary to maintain sufficient levels of protection against wild-type influenza infection. Similarly, adults who are particularly susceptible to repeated or serious influenza infection, such as, for example, health care workers, day care workers, family members of young children, the elderly, and individuals with compromised cardiopulmonary function may require multiple immunizations to establish and/or maintain protective immune responses. Levels of induced immunity can be monitored, for example, by measuring amounts of neutralizing secretory and serum antibodies, and dosages adjusted or vaccinations repeated as necessary to elicit and maintain desired levels of protection.

Optionally, the formulation for prophylactic administration of the influenza viruses also contains one or more adjuvants for enhancing the immune response to the influenza antigens. Suitable adjuvants include: complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, bacille Calmette-Guerin (BCG), *Corynebacterium parvum*, and the synthetic adjuvant QS-21.

If desired, prophylactic vaccine administration of influenza viruses can be performed in conjunction with administration of one or more immunostimulatory molecules Immunostimulatory molecules include various cytokines, lymphokines and chemokines with immunostimulatory, immunopotentiating, and pro-inflammatory activities, such as interleukins (e.g., IL-1, IL-2, IL-3, IL-4, IL-12, IL-13); growth factors (e.g., granulocyte-macrophage (GM)-colony stimulating factor (CSF)); and other immunostimulatory molecules, such as macrophage inflammatory factor, Flt3 ligand, B7.1; B7.2, etc. The immunostimulatory molecules can be administered in the same formulation as the influenza viruses, or can be administered separately. Either the protein (e.g., an HA and/or NA polypeptide of the invention, e.g., any of SEQ ID NO: 11-20, 27-32, or 39-44) or an expression vector comprising a nucleic acid (e.g., any of SEQ ID NO: 1-10, 21-26, 33-38, or 45) encoding the protein can be administered to produce an immunostimulatory effect.

The above described methods are useful for therapeutically and/or prophylactically treating a disease or disorder, typically influenza, by introducing a vector of the invention comprising a heterologous polynucleotide encoding a therapeutically or prophylactically effective HA and/or NA polypeptide (or peptide) or HA and/or NA RNA (e.g., an antisense RNA or ribozyme) into a population of target cells in vitro, ex vivo or in vivo. Typically, the polynucleotide encoding the polypeptide (or peptide), or RNA, of interest is operably linked to appropriate regulatory sequences as described above in the sections entitled "Expression Vectors" and "Additional

Expression Elements." Optionally, more than one heterologous coding sequence is incorporated into a single vector or virus. For example, in addition to a polynucleotide encoding a therapeutically or prophylactically active HA and/or NA polypeptide or RNA, the vector can also include additional therapeutic or prophylactic polypeptides, e.g., antigens, co-stimulatory molecules, cytokines, antibodies, etc., and/or markers, and the like.

Although vaccination of an individual with an attenuated influenza virus of a particular strain of a particular subgroup can induce cross-protection against influenza virus of different strains and/or subgroups, cross-protection can be enhanced, if desired, by vaccinating the individual with attenuated influenza virus from at least two strains, e.g., each of which represents a different subgroup. Additionally, vaccine combinations can optionally include mixes of pandemic vaccines (e.g., those against pandemic influenza strains such as various avian strains, see, e.g., the sequences herein, or other pandemic strains) and non-pandemic strains. Vaccine mixtures (or multiple vaccinations) can comprise components from human strains and/or non-human influenza strains (e.g., avian and human, etc.). Similarly, the attenuated influenza virus vaccines of this invention can optionally be combined with vaccines that induce protective immune responses against other infectious agents.

Polynucleotides of the Invention

It is well known in the art that the HA and NA polynucleotide segments of influenza viruses comprise both a coding region (encoding the ORF) and noncoding regions (NCRs), both 5' and 3' of the HA and NA coding sequence. An example of these NCRs are shown in SEQ ID NOS:1-9 (outside of the ORFs). It is also known that primers can be made to these NCRs to facilitate amplification of the entire HA and NA segments of influenza virus. (see, e.g., Hoffmann et al. Arch Virol. 2001 December; 146(12):2275-89). Further, it is known that the NCRs of the HA and NA of influenza may increase the efficiency of achieving reassortants. Therefore, the polynucleotide sequences of these NCRs (including fragments and variants (e.g., at least about 60%, or at least 70%, or at least 80%, or at least 90%, or at least about 91% or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 98.5%, or at least about 98.7%, or at least about 99%, or at least about 99.1%, or at least about 99.2%, or at least about 99.3%, or at least about 99.4%, or at least about 99.5%, or at least about 99.6% or at least about 99.7%, or at least about 99.8%, or at least about 99.9% identity) thereof) are within the scope of this invention. When amplifying the HA and NA segments of any pandemic strain, one could make and use polynucleotide primers to bind conserved (e.g., among related strains) regions of the HA and NA NCRs for amplification (e.g., by RT-PCR). In one embodiment, HA and NA polynucleotides of the invention include both the NCR and ORF of the HA and NA sequences (including fragments and variants (e.g., at least about 60%, or at least 70%, or at least 80%, or at least 90%, or at least about 91% or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 98.5%, or at least about 98.7%, or at least about 99%, or at least about 99.1%, or at least about 99.2%, or at least about 99.3%, or at least about 99.4%, or at least about 99.5%, or at least about 99.6% or at least about 99.7%, or at least about 99.8%, or at least about 99.9%) thereof) of pandemic virus strains. In alternative embodiments, the HA and NA polynucleotides of the invention exclude the NCR, but include the ORF (including fragments and variants (e.g., at least about 60%, or at least 70%, or at least 80%, or at least 90%, or at least about 91% or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 98.5%, or at least about 98.7%, or at least about 99%, or at least about 99.1%, or at least about 99.2%, or at least about 99.3%, or at least about 99.4%, or at least about 99.5%, or at least about 99.6% or at least about 99.7%, or at least about 99.8%, or at least about 99.9% thereof)) of the HA and NA sequences of pandemic virus strains (e.g., SEQ ID NOS: 1-9).

The HA and NA polynucleotides of the invention, e.g., SEQ ID NO:1 through SEQ ID NO:10, SEQ ID NO:21 through SEQ ID NO:26, SEQ ID NO:33 through SEQ ID NO:38, SEQ ID NO:45, and fragments thereof, are optionally used in a number of different capacities alternative to, or in addition to, the vaccines described above. Other exemplary uses are described herein for illustrative purpose and not as limitations on the actual range of uses. Different methods of construction, purification, and characterization of the nucleotide sequences of the invention are also described herein. In some embodiments, nucleic acids including one or more polynucleotide sequence of the invention are favorably used as probes for the detection of corresponding or related nucleic acids in a variety of contexts, such as in nucleic hybridization experiments, e.g., to find and/or characterize homologous influenza variants (e.g., homologues to the sequences herein, etc.) infecting other species or in different influenza outbreaks, etc. The probes can be either DNA or RNA molecules, such as restriction fragments of genomic or cloned DNA, cDNAs, PCR amplification products, transcripts, and oligonucleotides, and can vary in length from oligonucleotides as short as about 10 nucleotides in length to full length sequences or cDNAs in excess of 1 kb or more. For example, in some embodiments, a probe of the invention includes a polynucleotide sequence or subsequence selected, e.g., from among SEQ ID NO: 1 through SEQ ID NO: 10, SEQ ID NO:21 through SEQ ID NO:26, SEQ ID NO:33 through SEQ ID NO:38, SEQ ID NO:45, or sequences complementary thereto. Alternatively, polynucleotide sequences that are variants of one of the above-designated sequences are used as probes. Most typically, such variants include one or a few conservative nucleotide variations. For example, pairs (or sets) of oligonucleotides can be selected, in which the two (or more) polynucleotide sequences are conservative variations of each other, wherein one polynucleotide sequence corresponds identically to a first variant or and the other(s) corresponds identically to additional variants. Such pairs of oligonucleotide probes are particularly useful, e.g., for specific hybridization experiments to detect polymorphic nucleotides or to, e.g., detect homologous influenza HA and NA variants, e.g., homologous to the current HA and NA sequences, infecting other species or present in different (e.g., either temporally and/or geographically different) influenza outbreaks. In other applications, probes are selected that are more divergent, that is probes that are at least about 91% (or about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 98.5%, about 98.7%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, or about 99.6% or more about 99.7%, about 99.8%, about 99.9% or more) identical are selected.

The probes of the invention, e.g., as exemplified by sequences derived from the sequences herein, can also be used to identify additional useful polynucleotide sequences according to procedures routine in the art. In one set of embodiments, one or more probes, as described above, are utilized to screen libraries of expression products or chromosomal segments (e.g., expression libraries or genomic

libraries) to identify clones that include sequences identical to, or with significant sequence similarity to, e.g., one or more probe of the sequences herein, i.e., variants, homologues, etc. It will be understood that in addition to such physical methods as library screening, computer assisted bioinformatic approaches, e.g., BLAST and other sequence homology search algorithms, and the like, can also be used for identifying related polynucleotide sequences. Polynucleotide sequences identified in this manner are also a feature of the invention.

Oligonucleotide probes are optionally produced via a variety of methods well known to those skilled in the art. Most typically, they are produced by well known synthetic methods, such as the solid phase phosphoramidite triester method described by Beaucage and Caruthers (1981) *Tetrahedron Letts* 22(20):1859-1862, e.g., using an automated synthesizer, or as described in Needham-Van Devanter et al. (1984) *Nucl Acids Res,* 12:6159-6168. Oligonucleotides can also be custom made and ordered from a variety of commercial sources known to persons of skill. Purification of oligonucleotides, where necessary, is typically performed by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson and Regnier (1983) *J Chrom* 255:137-149. The sequence of the synthetic oligonucleotides can be verified using the chemical degradation method of Maxam and Gilbert (1980) in Grossman and Moldave (eds.) Academic Press, New York, *Methods in Enzymology* 65:499-560. Custom oligos can also easily be ordered from a variety of commercial sources known to persons of skill.

In other circumstances, e.g., relating to attributes of cells or organisms expressing the polynucleotides and polypeptides of the invention (e.g., those harboring virus comprising the sequences of the invention), probes that are polypeptides, peptides or antibodies are favorably utilized. For example, isolated or recombinant polypeptides, polypeptide fragments and peptides derived from any of the amino acid sequences of the invention (e.g., SEQ ID NO: 11-20, SEQ ID NO: 27-32, SEQ ID NO:39-44) and/or encoded by polynucleotide sequences of the invention, e.g., selected from SEQ ID NO: 1 through SEQ ID NO: 10, SEQ ID NO: 21 through SEQ ID NO: 26, SEQ ID NO:33 through SEQ ID NO:38, and SEQ ID NO:45 are favorably used to identify and isolate antibodies, e.g., from phage display libraries, combinatorial libraries, polyclonal sera, and the like. Polypeptide fragments of the inventions include a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, or at least 10 contiguous amino acid residues, or at least 15 contiguous amino acid residues, or at least 20 contiguous amino acid residues, or at least 25 contiguous amino acid residues, or at least 40 contiguous amino acid residues, or at least 50 contiguous amino acid residues, or at least 60 contiguous amino residues, or at least 70 contiguous amino acid residues, or at least contiguous 80 amino acid residues, or at least contiguous 90 amino acid residues, or at least contiguous 100 amino acid residues, or at least contiguous 125 amino acid residues, or at least 150 contiguous amino acid residues, or at least contiguous 175 amino acid residues, or at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues, or at least contiguous 350, or at least contiguous 400, or at least contiguous 450, or at least contiguous 500, or at least contiguous 550 amino acid residues of the amino acid sequence an HA or NA polypeptide of the invention (e.g., SEQ ID NOS: 11-20, SEQ ID NOS: 27-32, and SEQ ID NOS: 39-44). Polynucleotides encoding said polypeptide fragments and antibodies that specifically bind said polypeptides are also preferred embodiments of the invention.

Antibodies specific for any polypeptide sequence or subsequence, e.g., of SEQ ID NO: 11 through SEQ ID NO: 20, SEQ ID NO: 27 through SEQ ID NO: 32, and/or SEQ ID NO: 39 through SEQ ID NO: 44, and/or encoded by polynucleotide sequences of the invention, e.g., selected from SEQ ID NO: 1 through SEQ ID NO: 10, SEQ ID NO: 21 through SEQ ID NO: 26, SEQ ID NO: 33 through SEQ ID NO: 38, and SEQ ID NO:45 are likewise valuable as probes for evaluating expression products, e.g., from cells or tissues. In addition, antibodies are particularly suitable for evaluating expression of proteins comprising amino acid subsequences, e.g., of those given herein, or encoded by polynucleotides sequences of the invention, e.g., selected from those shown herein, in situ, in a tissue array, in a cell, tissue or organism, e.g., an organism infected by an unidentified influenza virus or the like. Antibodies can be directly labeled with a detectable reagent, or detected indirectly by labeling of a secondary antibody specific for the heavy chain constant region (i.e., isotype) of the specific antibody. Additional details regarding production of specific antibodies are provided below.

Diagnostic Assays

The nucleic acid sequences of the present invention can be used in diagnostic assays to detect influenza (and/or hemagglutinin and/or neuraminidase) in a sample, to detect hemagglutinin-like and/or neuraminidase-like sequences, and to detect strain differences in clinical isolates of influenza using either chemically synthesized or recombinant polynucleotide fragments, e.g., selected from the sequences herein. For example, fragments of the hemagglutinin and/or neuraminidase sequences comprising at least between 10 and 20 nucleotides can be used as primers to amplify nucleic acids using polymerase chain reaction (PCR) methods well known in the art (e.g., reverse transcription-PCR) and as probes in nucleic acid hybridization assays to detect target genetic material such as influenza RNA in clinical specimens.

The probes of the invention, e.g., as exemplified by unique subsequences selected from those given herein, can also be used to identify additional useful polynucleotide sequences (such as to characterize additional strains of influenza) according to procedures routine in the art. In one set of preferred embodiments, one or more probes, as described above, are utilized to screen libraries of expression products or cloned viral nucleic acids (i.e., expression libraries or genomic libraries) to identify clones that include sequences identical to, or with significant sequence identity to the sequences herein. In turn, each of these identified sequences can be used to make probes, including pairs or sets of variant probes as described above. It will be understood that in addition to such physical methods as library screening, computer assisted bioinformatic approaches, e.g., BLAST and other sequence homology search algorithms, and the like, can also be used for identifying related polynucleotide sequences.

The probes of the invention are particularly useful for detecting the presence and for determining the identity of influenza nucleic acids in cells, tissues or other biological samples (e.g., a nasal wash or bronchial lavage). For example, the probes of the invention are favorably utilized to determine whether a biological sample, such as a subject (e.g., a human subject) or model system (such as a cultured cell sample) has been exposed to, or become infected with influenza, or particular strain(s) of influenza. Detection of hybridization of the selected probe to nucleic acids originating in (e.g., isolated from) the biological sample or model system is indicative of exposure to or infection with the virus (or a related virus) from which the probe polynucleotide is selected.

It will be appreciated that probe design is influenced by the intended application. For example, where several allele-spe-

cific probe-target interactions are to be detected in a single assay, e.g., on a single DNA chip, it is desirable to have similar melting temperatures for all of the probes. Accordingly, the lengths of the probes are adjusted so that the melting temperatures for all of the probes on the array are closely similar (it will be appreciated that different lengths for different probes may be needed to achieve a particular T_m, where different probes have different GC contents). Although melting temperature is a primary consideration in probe design, other factors are optionally used to further adjust probe construction, such as selecting against primer self-complementarity and the like.

Vectors, Promoters and Expression Systems

The present invention includes recombinant constructs incorporating one or more of the nucleic acid sequences described herein. Such constructs optionally include a vector, for example, a plasmid, a cosmid, a phage, a virus, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), etc., into which one or more of the polynucleotide sequences of the invention, e.g., comprising any of SEQ ID NO: 1 through SEQ ID NO:10, SEQ ID NO:21 through SEQ ID NO:26, SEQ ID NO:33 through SEQ ID NO:38, SEQ ID NO:45 or a subsequence thereof etc., has been inserted, in a forward or reverse orientation. For example, the inserted nucleic acid can include a viral chromosomal sequence or cDNA including all or part of at least one of the polynucleotide sequences of the invention. In one embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

The polynucleotides of the present invention can be included in any one of a variety of vectors suitable for generating sense or antisense RNA, and optionally, polypeptide (or peptide) expression products (e.g., a hemagglutinin and/or neuraminidase molecule of the invention, or fragments thereof). Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adenovirus, adeno-associated virus, retroviruses and many others (e.g., pCDL). Any vector that is capable of introducing genetic material into a cell, and, if replication is desired, which is replicable in the relevant host can be used.

In an expression vector, the HA and/or NA polynucleotide sequence of interest is physically arranged in proximity and orientation to an appropriate transcription control sequence (e.g., promoter, and optionally, one or more enhancers) to direct mRNA synthesis. That is, the polynucleotide sequence of interest is operably linked to an appropriate transcription control sequence. Examples of such promoters include: LTR or SV40 promoter, *E. coli* lac or tip promoter, phage lambda P_L promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses.

A variety of promoters are suitable for use in expression vectors for regulating transcription of influenza virus genome segments. In certain embodiments, the cytomegalovirus (CMV) DNA dependent RNA Polymerase II (Pol II) promoter is utilized. If desired, e.g., for regulating conditional expression, other promoters can be substituted which induce RNA transcription under the specified conditions, or in the specified tissues or cells. Numerous viral and mammalian, e.g., human promoters are available, or can be isolated according to the specific application contemplated. For example, alternative promoters obtained from the genomes of animal and human viruses include such promoters as the adenovirus (such as Adenovirus 2), papilloma virus, hepatitis-B virus, polyoma virus, and Simian Virus 40 (SV40), and various retroviral promoters. Mammalian promoters include, among many others, the actin promoter, immunoglobulin promoters, heat-shock promoters, and the like.

Transcription is optionally increased by including an enhancer sequence. Enhancers are typically short, e.g., 10-500 bp, cis-acting DNA elements that act in concert with a promoter to increase transcription. Many enhancer sequences have been isolated from mammalian genes (hemoglobin, elastase, albumin, alpha-fetoprotein, and insulin), and eukaryotic cell viruses. The enhancer can be spliced into the vector at a position 5' or 3' to the heterologous coding sequence, but is typically inserted at a site 5' to the promoter. Typically, the promoter, and if desired, additional transcription enhancing sequences are chosen to optimize expression in the host cell type into which the heterologous DNA is to be introduced (Scharf et al. (1994) *Heat stress promoters and transcription factors Results Probl Cell Differ* 20:125-62; Kriegler et al. (1990) *Assembly of enhancers, promoters, and splice signals to control expression of transferred genes Methods in Enzymol* 185: 512-27). Optionally, the amplicon can also contain a ribosome binding site or an internal ribosome entry site (IRES) for translation initiation.

The vectors of the invention also favorably include sequences necessary for the termination of transcription and for stabilizing the mRNA, such as a polyadenylation site or a terminator sequence. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. In one embodiment, the SV40 polyadenylation signal sequences can provide a bidirectional polyadenylation site that insulates transcription of (+) strand mRNA molecules from the PolI promoter initiating replication of the (−) strand viral genome.

In addition, as described above, the expression vectors optionally include one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells, in addition to genes previously listed, markers such as dihydrofolate reductase or neomycin resistance are suitable for selection in eukaryotic cell culture.

The vector containing the appropriate nucleic acid sequence as described above, as well as an appropriate promoter or control sequence, can be employed to transform a host cell permitting expression of the protein. While the vectors of the invention can be replicated in bacterial cells, most frequently it will be desirable to introduce them into mammalian cells, e.g., Vero cells, BHK cells, MDCK cell, 293 cells, COS cells, or the like, for the purpose of expression.

As described elsewhere, the HA and NA sequences herein, in various embodiments, can be comprised within plasmids involved in plasmid-rescue reassortment. See, e.g., U.S. Application Nos. 60/420,708, filed Oct. 23, 2002; 60/574,117, filed May 24, 2004; 10/423,828, filed Apr. 25, 2003; 60/578,962, filed Jun. 12, 2004; and 10/870,690 filed Jun. 16, 2004; and US20020164770, which are incorporated by reference herein. For example, preferred expression vectors of the invention include, but are not limited to, vectors comprising pol I promoter and terminator sequences or vectors using both the pol I and pol II promoters "the polI/polII promoter system" (e.g., Zobel et al., Nucl. Acids Res. 1993, 21:3607; US20020164770; Neumann et al., Proc. Natl. Acad. Sci. USA 1999, 96:9345; Fodor et al., J. Virol. 1999, 73:9679; and US20030035814). The reassortants produced can include the HA and NA genes arranged with the 6 other influenza genes from the A/Ann Arbor/6/60 donor strain (and/or derivatives

and modifications thereof), the PR8 donor strain backbone, the A/Leningrad/17 donor strain backbone, etc. Other backbone strains are described, for example, in 20040137013 and 20030147916, which are incorporated by reference herein.

Additional Expression Elements

Most commonly, the genome segment encoding the influenza virus HA and/or NA protein includes any additional sequences necessary for its expression, including translation into a functional viral protein. In other situations, a minigene, or other artificial construct encoding the viral proteins, e.g., an HA and/or NA protein, can be employed. Again, in such case, it is often desirable to include specific initiation signals that aid in the efficient translation of the heterologous coding sequence. These signals can include, e.g., the ATG initiation codon and adjacent sequences. To insure translation of the entire insert, the initiation codon is inserted in the correct reading frame relative to the viral protein. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

If desired, polynucleotide sequences encoding additional expressed elements, such as signal sequences, secretion or localization sequences, and the like can be incorporated into the vector, usually, in-frame with the polynucleotide sequence of interest, e.g., to target polypeptide expression to a desired cellular compartment, membrane, or organelle, or to direct polypeptide secretion to the periplasmic space or into the cell culture media. Such sequences are known to those of skill, and include secretion leader peptides, organelle targeting sequences (e.g., nuclear localization sequences, ER retention signals, mitochondrial transit sequences), membrane localization/anchor sequences (e.g., stop transfer sequences, GPI anchor sequences), and the like.

Where translation of a polypeptide encoded by a nucleic acid sequence of the invention is desired, additional translation specific initiation signals can improve the efficiency of translation. These signals can include, e.g., an ATG initiation codon and adjacent sequences, an IRES region, etc. In some cases, for example, full-length cDNA molecules or chromosomal segments including a coding sequence incorporating, e.g., a polynucleotide sequence of the invention (e.g., as in the sequences herein), a translation initiation codon and associated sequence elements are inserted into the appropriate expression vector simultaneously with the polynucleotide sequence of interest. In such cases, additional translational control signals frequently are not required. However, in cases where only a polypeptide coding sequence, or a portion thereof, is inserted, exogenous translational control signals, including, e.g., an ATG initiation codon is often provided for expression of the relevant sequence. The initiation codon is put in the correct reading frame to ensure transcription of the polynucleotide sequence of interest. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, e.g., Scharf D. et al. (1994) *Results Probl Cell Differ* 20:125-62; Bittner et al. (1987) *Methods in Enzymol* 153:516-544).

Production of Recombinant Virus

Negative strand RNA viruses can be genetically engineered and recovered using a recombinant reverse genetics approach (see, e.g., U.S. Pat. No. 5,166,057 to Palese et al.). Such method was originally applied to engineer influenza viral genomes (Luytjes et al. (1989) Cell 59:1107-1113; Enami et al. (1990) *Proc. Natl. Acad. Sci. USA* 92:11563-11567), and has been successfully applied to a wide variety of segmented and nonsegmented negative strand RNA viruses, e.g., rabies (Schnell et al. (1994) *EMBO J.* 13: 4195-4203); VSV (Lawson et al. (1995) *Proc. Natl. Acad. Sci. USA* 92: 4477-4481); measles virus (Radecke et al. (1995) *EMBO J.* 14:5773-5784); rinderpest virus (Baron & Barrett (1997) *J. Virol.* 71: 1265-1271); human parainfluenza virus (Hoffman & Banerjee (1997) *J. Virol.* 71: 3272-3277; Dubin et al. (1997) *Virology* 235:323-332); SV5 (He et al. (1997) *Virology* 237:249-260); canine distemper virus (Gassen et al. (2000) *J. Virol.* 74:10737-44); and Sendai virus (Park et al. (1991) *Proc. Natl. Acad. Sci. USA* 88: 5537-5541; Kato et al. (1996) Genes to Cells 1:569-579). Those of skill in the art will be familiar with these and similar techniques to produce influenza virus comprising the HA and NA sequences of the invention. Recombinant influenza viruses produced according to such methods are also a feature of the invention, as are recombinant influenza virus comprising one or more nucleic acids and/or polypeptides of the invention.

Cell Culture and Expression Hosts

The present invention also relates to host cells that are introduced (transduced, transformed or transfected) with vectors of the invention, and the production of polypeptides of the invention by recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with a vector, such as an expression vector, of this invention. As described above, the vector can be in the form of a plasmid, a viral particle, a phage, etc. Examples of appropriate expression hosts include: bacterial cells, such as *E. coli, Streptomyces*, and *Salmonella typhimurium*; fungal cells, such as *Saccharomyces cerevisiae, Pichia pastoris*, and *Neurospora crassa*; or insect cells such as *Drosophila* and *Spodoptera frugiperda.*

Most commonly, mammalian cells are used to culture the HA and NA molecules of the invention. Suitable host cells for the replication of influenza virus include, e.g., Vero cells, BHK cells, MDCK cells, 293 cells and COS cells, including 293T cells, COS7 cells or the like. Commonly, co-cultures including two of the above cell lines, e.g., MDCK cells and either 293T or COS cells are employed at a ratio, e.g., of 1:1, to improve replication efficiency. Typically, cells are cultured in a standard commercial culture medium, such as Dulbecco's modified Eagle's medium supplemented with serum (e.g., 10% fetal bovine serum), or in serum free medium, under controlled humidity and CO_2 concentration suitable for maintaining neutral buffered pH (e.g., at pH between 7.0 and 7.2). Optionally, the medium contains antibiotics to prevent bacterial growth, e.g., penicillin, streptomycin, etc., and/or additional nutrients, such as L-glutamine, sodium pyruvate, non-essential amino acids, additional supplements to promote favorable growth characteristics, e.g., trypsin, β-mercaptoethanol, and the like.

The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the inserted polynucleotide sequences. The culture conditions, such as temperature, pH and the like, are typically those previously used with the particular host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, e.g., Freshney (1994) *Culture of Animal Cells, a Manual of Basic Technique,* 3rd edition, Wiley-Liss, New York and the references cited therein. Other helpful references include, e.g., Paul (1975) *Cell and Tissue Culture,* 5th ed., Livingston, Edinburgh; Adams (1980) *Laboratory Techniques in Biochemistry and Molecular Biology-Cell Culture for Biochemists*, Work and Burdon (eds.) Elsevier, Amsterdam. Additional details regarding tissue culture procedures of particular interest in the production of influenza

virus in vitro include, e.g., Merten et al. (1996) *Production of influenza virus in cell cultures for vaccine preparation*. in Cohen and Shafferman (eds.) *Novel Strategies in Design and Production of Vaccines*, which is incorporated herein in its entirety for all purposes. Additionally, variations in such procedures adapted to the present invention are readily determined through routine experimentation and will be familiar to those skilled in the art.

Cells for production of influenza virus (e.g., having the HA and/or NA sequences of the invention) can be cultured in serum-containing or serum free medium. In some cases, e.g., for the preparation of purified viruses, it is typically desirable to grow the host cells in serum free conditions. Cells can be cultured in small scale, e.g., less than 25 ml medium, culture tubes or flasks or in large flasks with agitation, in rotator bottles, or on microcarrier beads (e.g., DEAE-Dextran microcarrier beads, such as Dormacell, Pfeifer & Langen; Superbead, Flow Laboratories; styrene copolymer-tri-methylamine beads, such as Hillex, SoloHill, Ann Arbor) in flasks, bottles or reactor cultures. Microcarrier beads are small spheres (in the range of 100-200 microns in diameter) that provide a large surface area for adherent cell growth per volume of cell culture. For example a single liter of medium can include more than 20 million microcarrier beads providing greater than 8000 square centimeters of growth surface. For commercial production of viruses, e.g., for vaccine production, it is often desirable to culture the cells in a bioreactor or fermenter. Bioreactors are available in volumes from under 1 liter to in excess of 100 liters, e.g., Cyto3 Bioreactor (Osmonics, Minnetonka, Minn.); NBS bioreactors (New Brunswick Scientific, Edison, N.J.); laboratory and commercial scale bioreactors from B. Braun Biotech International (B. Braun Biotech, Melsungen, Germany).

Regardless of the culture volume, in many desired aspects of the current invention, it is important that the cultures be maintained at an appropriate temperature, to insure efficient recovery of recombinant and/or reassortant influenza virus using temperature dependent multi plasmid systems (see, e.g., Multi-Plasmid System for the Production of Influenza Virus, U.S. Application No. 60/420,708, filed Oct. 23, 2002, U.S. application Ser. No. 10/423,828, filed Apr. 25, 2003, and U.S. Application No. 60/574,117, filed May 24, 2004), heating of virus solutions for filtration, etc. Typically, a regulator, e.g., a thermostat, or other device for sensing and maintaining the temperature of the cell culture system and/or other solution, is employed to insure that the temperature is at the correct level during the appropriate period (e.g., virus replication, etc.).

In some embodiments herein (e.g., wherein reassorted viruses are to be produced from segments on vectors) vectors comprising influenza genome segments are introduced (e.g., transfected) into host cells according to methods well known in the art for introducing heterologous nucleic acids into eukaryotic cells, including, e.g., calcium phosphate co-precipitation, electroporation, microinjection, lipofection, and transfection employing polyamine transfection reagents. For example, vectors, e.g., plasmids, can be transfected into host cells, such as COS cells, 293T cells or combinations of COS or 293T cells and MDCK cells, using the polyamine transfection reagent TransIT-LT1 (Mirus) according to the manufacturer's instructions in order to produce reassorted viruses, etc. Thus, in one example, approximately 1 μg of each vector is introduced into a population of host cells with approximately 2 μl of TransIT-LT1 diluted in 160 μl medium, preferably serum-free medium, in a total volume of 200 μl. The DNA:transfection reagent mixtures are incubated at room temperature for 45 minutes followed by addition of 800 μl of medium. The transfection mixture is added to the host cells, and the cells are cultured as described via other methods well known to those skilled in the art. Accordingly, for the production of recombinant or reassortant viruses in cell culture, vectors incorporating each of the 8 genome segments, (PB2, PB1, PA, NP, M, NS, HA and NA, e.g., of the invention) are mixed with approximately 20 μl TransIT-LT1 and transfected into host cells. Optionally, serum-containing medium is replaced prior to transfection with serum-free medium, e.g., Opti-MEM I, and incubated for 4-6 hours.

Alternatively, electroporation can be employed to introduce such vectors incorporating influenza genome segments into host cells. For example, plasmid vectors incorporating an influenza A or influenza B virus are favorably introduced into Vero cells using electroporation according to the following procedure. In brief, approximately 5×10^6 Vero cells, e.g., grown in Modified Eagle's Medium (MEM) supplemented with 10% Fetal Bovine Serum (FBS) are resuspended in 0.4 ml OptiMEM and placed in an electroporation cuvette. Twenty micrograms of DNA in a volume of up to 25 μl is added to the cells in the cuvette, which is then mixed gently by tapping. Electroporation is performed according to the manufacturer's instructions (e.g., BioRad Gene Pulser II with Capacitance Extender Plus connected) at 300 volts, 950 microFarads with a time constant of between 28-33 msec. The cells are remixed by gently tapping and approximately 1-2 minutes following electroporation 0.7 ml MEM with 10% FBS is added directly to the cuvette. The cells are then transferred to two wells of a standard 6 well tissue culture dish containing 2 ml MEM, 10% FBS. The cuvette is washed to recover any remaining cells and the wash suspension is divided between the two wells. Final volume is approximately 3.5 mL. The cells are then incubated under conditions permissive for viral growth, e.g., at approximately 33° C. for cold adapted strains.

In mammalian host cells, a number of expression systems, such as viral-based systems, can be utilized. In cases where an adenovirus is used as an expression vector, a coding sequence is optionally ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing the polypeptides of interest in infected host cells (Logan and Shenk (1984) *Proc Natl Acad Sci* 81:3655-3659). In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

A host cell strain is optionally chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing, which cleaves a precursor form into a mature form, of the protein is sometimes important for correct insertion, folding and/or function. Additionally proper location within a host cell (e.g., on the cell surface) is also important. Different host cells such as COS, CHO, BHK, MDCK, 293, 293T, COS7, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and can be chosen to ensure the correct modification and processing of the current introduced, foreign protein.

For long-term, high-yield production of recombinant proteins encoded by, or having subsequences encoded by, the polynucleotides of the invention, stable expression systems are optionally used. For example, cell lines, stably expressing a polypeptide of the invention, are transfected using expres-

sion vectors that contain viral origins of replication or endogenous expression elements and a selectable marker gene. For example, following the introduction of the vector, cells are allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Thus, resistant clumps of stably transformed cells, e.g., derived from single cell type, can be proliferated using tissue culture techniques appropriate to the cell type.

Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The cells expressing said protein can be sorted, isolated and/or purified. The protein or fragment thereof produced by a recombinant cell can be secreted, membrane-bound, or retained intracellularly, depending on the sequence (e.g., depending upon fusion proteins encoding a membrane retention signal or the like) and/or the vector used.

Expression products corresponding to the nucleic acids of the invention can also be produced in non-animal cells such as plants, yeast, fungi, bacteria and the like. In addition to Sambrook, Berger and Ausubel, all infra, details regarding cell culture can be found in Payne et al. (1992) *Plant Cell and Tissue Culture in Liquid Systems* John Wiley & Sons, Inc. New York, N.Y.; Gamborg and Phillips (eds.) (1995) *Plant Cell, Tissue and Organ Culture*; Fundamental Methods Springer Lab Manual, Springer-Verlag (Berlin Heidelberg New York) and Atlas and Parks (eds.) *The Handbook of Microbiological Media* (1993) CRC Press, Boca Raton, Fla.

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the expressed product. For example, when large quantities of a polypeptide or fragments thereof are needed for the production of antibodies, vectors that direct high-level expression of fusion proteins that are readily purified are favorably employed. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the coding sequence of interest, e.g., sequences comprising those found herein, etc., can be ligated into the vector in-frame with sequences for the amino-terminal translation initiating methionine and the subsequent 7 residues of beta-galactosidase producing a catalytically active beta galactosidase fusion protein; pIN vectors (Van Heeke & Schuster (1989) *J Biol Chem* 264:5503-5509); pET vectors (Novagen, Madison Wis.); and the like. Similarly, in the yeast *Saccharomyces cerevisiae* a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH can be used for production of the desired expression products. For reviews, see Ausubel, infra, and Grant et al., (1987); *Methods in Enzymology* 153: 516-544.

Nucleic Acid Hybridization

Comparative hybridization can be used to identify nucleic acids (e.g., SEQ ID NO: 1-10, SEQ ID NO: 21-26, SEQ ID NO:33-38, SEQ ID NO:45) of the invention, including conservative variations of nucleic acids of the invention. This comparative hybridization method is a preferred method of distinguishing nucleic acids of the invention. In addition, target nucleic acids which hybridize to the nucleic acids represented by, e.g., those shown herein under high, ultra-high and ultra-ultra-high stringency conditions are features of the invention. Examples of such nucleic acids include those with one or a few silent or conservative nucleic acid substitutions as compared to a given nucleic acid sequence.

A test target nucleic acid is said to specifically hybridize to a probe nucleic acid when it hybridizes at least one-half as well to the probe as to the perfectly matched complementary target, i.e., with a signal to noise ratio at least one-half as high as hybridization of the probe and target under conditions in which a perfectly matched probe binds to a perfectly matched complementary target with a signal to noise ratio that is at least about 5×-10× as high as that observed for hybridization to any of the unmatched target nucleic acids.

Nucleic acids "hybridize" when they associate, typically in solution. Nucleic acids hybridize due to a variety of well-characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. Numerous protocols for nucleic acid hybridization are well known in the art. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes* part I chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," (Elsevier, New York), as well as in Ausubel, Sambrook, and Berger and Kimmel, all below. Hames and Higgins (1995) *Gene Probes* 1 IRL Press at Oxford University Press, Oxford, England, (Hames and Higgins 1) and Hames and Higgins (1995) *Gene Probes* 2 IRL Press at Oxford University Press, Oxford, England (Hames and Higgins 2) provide details on the synthesis, labeling, detection and quantification of DNA and RNA, including oligonucleotides.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42° C., with the hybridization being carried out overnight. An example of stringent wash conditions comprises a 0.2×SSC wash at 65° C. for 15 minutes (see, Sambrook, infra for a description of SSC buffer and other nucleic acid hybridization parameters). Often the high stringency wash is preceded by a low stringency wash to remove background probe signal. An example low stringency wash is 2×SSC at 40° C. for 15 minutes. In general, a signal to noise ratio of 5× (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

After hybridization, unhybridized nucleic acids can be removed by a series of washes, the stringency of which can be adjusted depending upon the desired results. Low stringency washing conditions (e.g., using higher salt and lower temperature) increase sensitivity, but can produce nonspecific hybridization signals and high background signals. Higher stringency conditions (e.g., using lower salt and higher temperature that is closer to the T_m) lower the background signal, typically with primarily the specific signal remaining. See, also, Rapley, R. and Walker, J. M. eds., *Molecular Biomethods Handbook* (Humana Press, Inc. 1998).

"Stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993), supra, and in Hames and Higgins, 1 and 2. Stringent hybridization and wash conditions can easily be determined empirically for any test nucleic acid. For example, in determining highly stringent hybridization and wash conditions, the hybridization and wash conditions are gradually increased (e.g., by increasing temperature, decreasing salt concentration, increasing detergent concentration and/or increasing the concentration of organic solvents such

as formalin in the hybridization or wash), until a selected set of criteria is met. For example, the hybridization and wash conditions are gradually increased until a probe binds to a perfectly matched complementary target with a signal to noise ratio that is at least 5× as high as that observed for hybridization of the probe to an unmatched target.

In general, a signal to noise ratio of at least 2× (or higher, e.g., at least 5×, 10×, 20×, 50×, 100×, or more) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Detection of at least stringent hybridization between two sequences in the context of the present invention indicates relatively strong structural similarity to, e.g., the nucleic acids of the present invention provided in the sequence listings herein.

"Very stringent" conditions are selected to be equal to the thermal melting point (T_m) for a particular probe. The T_m, is the temperature (under defined ionic strength and pH) at which 50% of the test sequence hybridizes to a perfectly matched probe. For the purposes of the present invention, generally, "highly stringent" hybridization and wash conditions are selected to be about 5° C. lower than the T_m for the specific sequence at a defined ionic strength and pH (as noted below, highly stringent conditions can also be referred to in comparative terms). Target sequences that are closely related or identical to the nucleotide sequence of interest (e.g., "probe") can be identified under stringent or highly stringent conditions. Lower stringency conditions are appropriate for sequences that are less complementary.

"Ultra high-stringency" hybridization and wash conditions are those in which the stringency of hybridization and wash conditions are increased until the signal to noise ratio for binding of a probe to a perfectly matched complementary target nucleic acid is at least 10× as high as that observed for hybridization to any unmatched target nucleic acids. A target nucleic acid which hybridizes to a probe under such conditions, with a signal to noise ratio of at least one-half that of the perfectly matched complementary target nucleic acid is said to bind to the probe under ultra-high stringency conditions.

In determining stringent or highly stringent hybridization (or even more stringent hybridization) and wash conditions, the hybridization and wash conditions are gradually increased (e.g., by increasing temperature, decreasing salt concentration, increasing detergent concentration and/or increasing the concentration of organic solvents, such as formamide, in the hybridization or wash), until a selected set of criteria are met. For example, the hybridization and wash conditions are gradually increased until a probe comprising one or more polynucleotide sequences of the invention, e.g., sequences or unique subsequences selected from those given herein (e.g., SEQ ID NO: 1-10, 21-26, 33-38, SEQ ID NO:45) and/or complementary polynucleotide sequences, binds to a perfectly matched complementary target (again, a nucleic acid comprising one or more nucleic acid sequences or subsequences selected from those given herein and/or complementary polynucleotide sequences thereof), with a signal to noise ratio that is at least 2× (and optionally 5×, 10×, or 100× or more) as high as that observed for hybridization of the probe to an unmatched target (e.g., a polynucleotide sequence comprising one or more sequences or subsequences selected from known influenza sequences present in public databases such as GenBank at the time of filing, and/or complementary polynucleotide sequences thereof), as desired.

Using the polynucleotides of the invention, or subsequences thereof, novel target nucleic acids can be obtained; such target nucleic acids are also a feature of the invention. For example, such target nucleic acids include sequences that hybridize under stringent conditions to a unique oligonucleotide probe corresponding to any of the polynucleotides of the invention, e.g., SEQ ID NO: 1-10, 21-26, 33-38, 45).

Similarly, even higher levels of stringency can be determined by gradually increasing the hybridization and/or wash conditions of the relevant hybridization assay. For example, those in which the stringency of hybridization and wash conditions are increased until the signal to noise ratio for binding of the probe to the perfectly matched complementary target nucleic acid is at least 10×, 20×, 50×, 100×, or 500× or more as high as that observed for hybridization to any unmatched target nucleic acids. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radioactive label, or the like. A target nucleic acid which hybridizes to a probe under such conditions, with a signal to noise ratio of at least one-half that of the perfectly matched complementary target nucleic acid is said to bind to the probe under ultra-ultra-high stringency conditions and are also features of the invention.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

Cloning, Mutagenesis and Expression of Biomolecules of Interest

General texts which describe molecular biological techniques, which are applicable to the present invention, such as cloning, mutation, cell culture and the like, include Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology* volume 152 Academic Press, Inc., San Diego, Calif. (Berger); Sambrook et al., *Molecular Cloning—A Laboratory Manual* (3rd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2000 ("Sambrook") and *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2002) ("Ausubel")). These texts describe mutagenesis, the use of vectors, promoters and many other relevant topics related to, e.g., the generation of HA and/or NA molecules, etc.

Various types of mutagenesis are optionally used in the present invention, e.g., to produce and/or isolate, e.g., novel or newly isolated HA and/or NA molecules and/or to further modify/mutate the polypeptides (e.g., HA and NA molecules as in SEQ ID NO: 11-20 or 27-32 or 39-44) of the invention. They include but are not limited to site-directed, random point mutagenesis, homologous recombination (DNA shuffling), mutagenesis using uracil containing templates, oligonucleotide-directed mutagenesis, phosphorothioate-modified DNA mutagenesis, mutagenesis using gapped duplex DNA or the like. Additional suitable methods include point mismatch repair, mutagenesis using repair-deficient host strains, restriction-selection and restriction-purification, deletion mutagenesis, mutagenesis by total gene synthesis, double-strand break repair, and the like. Mutagenesis, e.g., involving chimeric constructs, is also included in the present invention. In one embodiment, mutagenesis can be guided by known information of the naturally occurring molecule or altered or mutated naturally occurring molecule, e.g., sequence, sequence comparisons, physical properties, crystal structure or the like.

The above texts and examples found herein describe these procedures as well as the following publications (and references cited within): Sieber, et al., *Nature Biotechnology*, 19:456-460 (2001); Ling et al., *Approaches to DNA mutagen-*

esis: an overview, Anal Biochem 254(2): 157-178 (1997); Dale et al., Oligonucleotide-directed random mutagenesis using the phosphorothioate method, Methods Mol Biol 57:369-374 (1996); I. A. Lorimer, I. Pastan, Nucleic Acids Res 23, 3067-8 (1995); W. P. C. Stemmer, Nature 370, 389-91 (1994); Arnold, Protein engineering for unusual environments, Current Opinion in Biotechnology 4:450-455 (1993); Bass et al., Mutant Trp repressors with new DNA-binding specificities, Science 242:240-245 (1988); Fritz et al., Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro, Nucl Acids Res 16: 6987-6999 (1988); Kramer et al., Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations, Nucl Acids Res 16: 7207 (1988); Sakamar and Khorana, Total synthesis and expression of a gene for the a-subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin), Nucl Acids Res 14: 6361-6372 (1988); Sayers et al., Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis, Nucl Acids Res 16:791-802 (1988); Sayers et al., Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide, (1988) Nucl Acids Res 16: 803-814; Carter, Improved oligonucleotide-directed mutagenesis using M13 vectors, Methods in Enzymol 154: 382-403 (1987); Kramer & Fritz Oligonucleotide-directed construction of mutations via gapped duplex DNA, Methods in Enzymol 154:350-367 (1987); Kunkel, The efficiency of oligonucleotide directed mutagenesis, in Nucleic Acids & Molecular Biology (Eckstein, F. and Lilley, D. M. J. eds., Springer Verlag, Berlin)) (1987); Kunkel et al., Rapid and efficient site-specific mutagenesis without phenotypic selection, Methods in Enzymol 154, 367-382 (1987); Zoller & Smith, Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template, Methods in Enzymol 154:329-350 (1987); Carter, Site-directed mutagenesis, Biochem J 237:1-7 (1986); Eghtedarzadeh & Henikoff, Use of oligonucleotides to generate large deletions, Nucl Acids Res 14: 5115 (1986); Mandecki, Oligonucleotide-directed double-strand break repair in plasmids of Escherichia coli: a method for site-specific mutagenesis, Proc Natl Acad Sci USA, 83:7177-7181 (1986); Nakamaye & Eckstein, Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis, Nucl Acids Res 14: 9679-9698 (1986); Wells et al., Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin, Phil Trans R Soc Lond A 317: 415-423 (1986); Botstein & Shortle, Strategies and applications of in vitro mutagenesis, Science 229:1193-1201 (1985); Carter et al., Improved oligonucleotide site-directed mutagenesis using M13 vectors, Nucl Acids Res 13: 4431-4443 (1985); Grundström et al., Oligonucleotide-directed mutagenesis by microscale 'shot-gun' gene synthesis, Nucl Acids Res 13: 3305-3316 (1985); Kunkel, Rapid and efficient site-specific mutagenesis without phenotypic selection, Proc Natl Acad Sci USA 82:488-492 (1985); Smith, In vitro mutagenesis, Ann Rev Genet. 19:423-462 (1985); Taylor et al., The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA, Nucl Acids Res 13: 8749-8764 (1985); Taylor et al., The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA, Nucl Acids Res 13: 8765-8787 (1985); Wells et al., Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites, Gene 34:315-323 (1985); Kramer et al., The gapped duplex DNA approach to oligonucleotide-directed mutation construction, Nucl Acids Res 12: 9441-9456 (1984); Kramer et al., Point Mismatch Repair, Cell 38:879-887 (1984); Nambiar et al., Total synthesis and cloning of a gene coding for the ribonuclease S protein, Science 223: 1299-1301 (1984); Zoller & Smith, Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors, Methods in Enzymol 100:468-500 (1983); and Zoller & Smith, Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment, Nucl Acids Res 10:6487-6500 (1982). Additional details on many of the above methods can be found in Methods in Enzymol Volume 154, which also describes useful controls for trouble-shooting problems with various mutagenesis, gene isolation, expression, and other methods.

Oligonucleotides, e.g., for use in mutagenesis of the present invention, e.g., mutating libraries of the HA and/or NA molecules of the invention, or altering such, are typically synthesized chemically according to the solid phase phosphoramidite triester method described by Beaucage and Caruthers, *Tetrahedron Letts* 22(20):1859-1862, (1981) e.g., using an automated synthesizer, as described in Needham-VanDevanter et al., *Nucleic Acids Res,* 12:6159-6168 (1984).

In addition, essentially any nucleic acid can be custom or standard ordered from any of a variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com), The Great American Gene Company (www.genco.com), ExpressGen Inc. (www.expressgen-.com), Operon Technologies Inc. (Alameda, Calif.) and many others. Similarly, peptides and antibodies can be custom ordered from any of a variety of sources, such as PeptidoGenic (available at pkim@ccnet.com), HTI Bio-products, Inc. (www.htibio.com), BMA Biomedicals Ltd. (U.K.), Bio-.Synthesis, Inc., and many others.

The present invention also relates to host cells and organisms comprising a HA and/or NA molecule or other polypeptide and/or nucleic acid of the invention, e.g., SEQ ID NOS: 1-45. Host cells are genetically engineered (e.g., transformed, transduced or transfected) with the vectors of this invention, which can be, for example, a cloning vector or an expression vector. The vector can be, for example, in the form of a plasmid, a bacterium, a virus, a naked polynucleotide, or a conjugated polynucleotide. The vectors are introduced into cells and/or microorganisms by standard methods including electroporation (see, From et al., *Proc Natl Acad Sci USA* 82, 5824 (1985), infection by viral vectors, high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., *Nature* 327, 70-73 (1987)). Berger, Sambrook, and Ausubel provide a variety of appropriate transformation methods. See, above.

Several well-known methods of introducing target nucleic acids into bacterial cells are available, any of which can be used in the present invention. These include: fusion of the recipient cells with bacterial protoplasts containing the DNA, electroporation, projectile bombardment, and infection with viral vectors, etc. Bacterial cells can be used to amplify the number of plasmids containing DNA constructs of this invention. The bacteria are grown to log phase and the plasmids within the bacteria can be isolated by a variety of methods known in the art (see, for instance, Sambrook). In addition, a plethora of kits are commercially available for the purification of plasmids from bacteria, (see, e.g., EasyPrep™, FlexiPrep™, both from Pharmacia Biotech; StrataClean™, from Stratagene; and, QIAprep™ from Qiagen). The isolated and purified plasmids are then further manipulated to produce other plasmids, used to transfect cells or incorporated into related vectors to infect organisms. Typical vectors contain

transcription and translation terminators, transcription and translation initiation sequences, and promoters useful for regulation of the expression of the particular target nucleic acid. The vectors optionally comprise generic expression cassettes containing at least one independent terminator sequence, sequences permitting replication of the cassette in eukaryotes, or prokaryotes, or both, (e.g., shuttle vectors) and selection markers for both prokaryotic and eukaryotic systems. Vectors are suitable for replication and integration in prokaryotes, eukaryotes, or optionally both. See, Giliman & Smith, *Gene* 8:81 (1979); Roberts, et al., *Nature,* 328:731 (1987); Schneider, B., et al., *Protein Expr Purif* 6435:10 (1995); Ausubel, Sambrook, Berger (all supra). A catalogue of Bacteria and Bacteriophages useful for cloning is provided, e.g., by the ATCC, e.g., *The ATCC Catalogue of Bacteria and Bacteriophage* (1992) Gherna et al. (eds.) published by the ATCC. Additional basic procedures for sequencing, cloning and other aspects of molecular biology and underlying theoretical considerations are also found in Watson et al. (1992) *Recombinant DNA Second Edition* Scientific American Books, NY. See, above. Further vectors useful with the sequences herein are illustrated above in the section concerning production of influenza virus for vaccines and the references cited therein.

Polypeptide Production and Recovery

Following transduction of a suitable host cell line or strain and growth of the host cells to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. In some embodiments, a secreted polypeptide product, e.g., a HA and/or NA polypeptide as in a secreted fusion protein form, etc., is then recovered from the culture medium. In other embodiments, a virus particle containing a HA and/or a NA polypeptide of the invention is produced from the cell. Alternatively, cells can be harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Eukaryotic or microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art. Additionally, cells expressing a HA and/or a NA polypeptide product of the invention can be utilized without separating the polypeptide from the cell. In such situations, the polypeptide of the invention is optionally expressed on the cell surface and is examined thus (e.g., by having HA and/or NA molecules (or fragments thereof, e.g., comprising fusion proteins or the like) on the cell surface bind antibodies, etc. Such cells are also features of the invention.

Expressed polypeptides can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography (e.g., using any of the tagging systems known to those skilled in the art), hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as desired, in completing configuration of the mature protein. Also, high performance liquid chromatography (HPLC) can be employed in the final purification steps. In addition to the references noted herein, a variety of purification methods are well known in the art, including, e.g., those set forth in Sandana (1997) *Bioseparation of Proteins*, Academic Press, Inc.; and Bollag et al. (1996) *Protein Methods, 2nd Edition* Wiley-Liss, NY; Walker (1996) *The Protein Protocols Handbook* Humana Press, NJ, Harris and Angal (1990) *Protein Purification Applications: A Practical Approach* IRL Press at Oxford, Oxford, England; Harris and Angal *Protein Purification Methods: A Practical Approach* IRL Press at Oxford, Oxford, England; Scopes (1993) *Protein Purification: Principles and Practice* 3rd *Edition* Springer Verlag, NY; Janson and Ryden (1998) *Protein Purification: Principles, High Resolution Methods and Applications, Second Edition* Wiley-VCH, NY; and Walker (1998) *Protein Protocols on CD-ROM* Humana Press, NJ.

When the expressed polypeptides of the invention are produced in viruses, the viruses are typically recovered from the culture medium, in which infected (transfected) cells have been grown. Typically, crude medium is clarified prior to concentration of influenza viruses. Common methods include ultrafiltration, adsorption on barium sulfate and elution, and centrifugation. For example, crude medium from infected cultures can first be clarified by centrifugation at, e.g., 1000-2000×g for a time sufficient to remove cell debris and other large particulate matter, e.g., between 10 and 30 minutes. Optionally, the clarified medium supernatant is then centrifuged to pellet the influenza viruses, e.g., at 15,000×g, for approximately 3-5 hours. Following resuspension of the virus pellet in an appropriate buffer, such as STE (0.01M Tris-HCl; 0.15 M NaCl; 0.0001M EDTA) or phosphate buffered saline (PBS) at pH 7.4, the virus is concentrated by density gradient centrifugation on sucrose (60%-12%) or potassium tartrate (50%-10%). Either continuous or step gradients, e.g., a sucrose gradient between 12% and 60% in four 12% steps, are suitable. The gradients are centrifuged at a speed, and for a time, sufficient for the viruses to concentrate into a visible band for recovery. Alternatively, and for most large-scale commercial applications, virus is elutriated from density gradients using a zonal-centrifuge rotor operating in continuous mode. Additional details sufficient to guide one of skill through the preparation of influenza viruses from tissue culture are provided, e.g., in Furminger. *Vaccine Production*, in Nicholson et al. (eds.) *Textbook of Influenza* pp. 324-332; Merten et al. (1996) *Production of influenza virus in cell cultures for vaccine preparation*, in Cohen & Shafferman (eds.) *Novel Strategies in Design and Production of Vaccines* pp. 141-151, and U.S. Pat. No. 5,690,937. If desired, the recovered viruses can be stored at −80° C. in the presence of sucrose-phosphate-glutamate (SPG) as a stabilizer

Alternatively, cell-free transcription/translation systems can be employed to produce polypeptides comprising an amino acid sequence or subsequence of, e.g., the sequences given herein such as SEQ ID NOS: 11-20 or 27-32 or 39-44, or encoded by the polynucleotide sequences of the invention, e.g., SEQ ID NOS: 1-10 or 21-26 or 33-38 or 45. A number of suitable in vitro transcription and translation systems are commercially available. A general guide to in vitro transcription and translation protocols is found in Tymms (1995) *In vitro Transcription and Translation Protocols: Methods in Molecular Biology* Volume 37, Garland Publishing, NY.

In addition, the polypeptides, or subsequences thereof, e.g., subsequences comprising antigenic peptides, can be produced manually or by using an automated system, by direct peptide synthesis using solid-phase techniques (see, Stewart et al. (1969) *Solid-Phase Peptide Synthesis*, WH Freeman Co, San Francisco; Merrifield J (1963) *J Am Chem Soc* 85:2149-2154). Exemplary automated systems include the Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.). If desired, subsequences can be chemically synthesized separately, and combined using chemical methods to provide full-length polypeptides.

Modified Amino Acids

Expressed polypeptides of the invention can contain one or more modified amino acids. The presence of modified amino acids can be advantageous in, for example, (a) increasing polypeptide serum half-life, (b) reducing/increasing polypeptide antigenicity, (c) increasing polypeptide storage stability, etc Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production (e.g., N-linked glycosylation at N-X-S/T motifs during expression in mammalian cells) or modified by synthetic means (e.g., via PEGylation).

Non-limiting examples of a modified amino acid include a glycosylated amino acid, a sulfated amino acid, a prenlyated (e.g., farnesylated, geranylgeranylated) amino acid, an acetylated amino acid, an acylated amino acid, a PEG-ylated amino acid, a biotinylated amino acid, a carboxylated amino acid, a phosphorylated amino acid, and the like, as well as amino acids modified by conjugation to, e.g., lipid moieties or other organic derivatizing agents. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature. Example protocols are found in Walker (1998) *Protein Protocols on CD-ROM* Human Press, Towata, N.J.

Fusion Proteins

The present invention also provides fusion proteins comprising fusions of the sequences of the invention (e.g., encoding HA and/or NA polypeptides as exampled by SEQ ID NOS: 11-20, 27-32, and 39-44) or fragments thereof with, e.g., immunoglobulins (or portions thereof), sequences encoding, e.g., GFP (green fluorescent protein), or other similar markers, etc. Nucleotide sequences encoding such fusion proteins are another aspect of the invention. Fusion proteins of the invention are optionally used for, e.g., similar applications (including, e.g., therapeutic, prophylactic, diagnostic, experimental, etc. applications as described herein) as the non-fusion proteins of the invention. In addition to fusion with immunoglobulin sequences and marker sequences, the proteins of the invention are also optionally fused with, e.g., sequences which allow sorting of the fusion proteins and/or targeting of the fusion proteins to specific cell types, regions, etc.

Antibodies

The polypeptides of the invention can be used to produce antibodies specific for the polypeptides given herein and/or polypeptides encoded by the polynucleotides of the invention, e.g., those shown herein, and conservative variants thereof. Antibodies specific for the above mentioned polypeptides are useful, e.g., for diagnostic and therapeutic purposes, e.g., related to the activity, distribution, and expression of target polypeptides.

Antibodies specific for the polypeptides of the invention can be generated by methods well known in the art. Such antibodies can include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library.

Polypeptides do not require biological activity for antibody production (e.g., full length functional hemagglutinin or neuraminidase is not required). However, the polypeptide or oligopeptide must be antigenic. Peptides used to induce specific antibodies typically have an amino acid sequence of at least about 4 amino acids, and often at least 5 or 10 amino acids. Short stretches of a polypeptide can be fused with another protein, such as keyhole limpet hemocyanin, and antibody produced against the chimeric molecule.

Numerous methods for producing polyclonal and monoclonal antibodies are known to those of skill in the art, and can be adapted to produce antibodies specific for the polypeptides of the invention, and/or encoded by the polynucleotide sequences of the invention, etc. See, e.g., Coligan (1991) *Current Protocols in Immunology* Wiley/Greene, N.Y.; Paul (ed.) (1998) *Fundamental Immunology, Fourth Edition*, Lippincott-Raven, Lippincott Williams & Wilkins; Harlow and Lane (1989) *Antibodies: A Laboratory Manual* Cold Spring Harbor Press, NY; Stites et al. (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, Calif., and references cited therein; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed.) Academic Press, New York, N.Y.; and Kohler and Milstein (1975) Nature 256: 495-497. Other suitable techniques for antibody preparation include selection of libraries of recombinant antibodies in phage or similar vectors. See, Huse et al. (1989) *Science* 246: 1275-1281; and Ward, et al. (1989) *Nature* 341: 544-546. Specific monoclonal and polyclonal antibodies and antisera will usually bind with a K_D of, e.g., at least about 0.1 μM, at least about 0.01 μM or better, and, typically and at least about 0.001 μM or better.

For certain therapeutic applications, humanized antibodies are desirable. Detailed methods for preparation of chimeric (humanized) antibodies can be found in U.S. Pat. No. 5,482,856. Additional details on humanization and other antibody production and engineering techniques can be found in Borrebaeck (ed.) (1995) *Antibody Engineering, 2nd Edition* Freeman and Company, NY (Borrebaeck); McCafferty et al. (1996) *Antibody Engineering, A Practical Approach* IRL at Oxford Press, Oxford, England (McCafferty), and Paul (1995) *Antibody Engineering Protocols* Humana Press, Towata, N.J. (Paul). Additional details regarding specific procedures can be found, e.g., in Ostberg et al. (1983), *Hybridoma* 2: 361-367, Ostberg, U.S. Pat. No. 4,634,664, and Engelman et al., U.S. Pat. No. 4,634,666.

Defining Polypeptides by Immunoreactivity

Because the polypeptides of the invention provide a variety of new polypeptide sequences (e.g., comprising HA and NA molecules), the polypeptides also provide new structural features which can be recognized, e.g., in immunological assays. The generation of antisera which specifically bind the polypeptides of the invention, as well as the polypeptides which are bound by such antisera, are features of the invention.

For example, the invention includes polypeptides (e.g., HA and NA molecules) that specifically bind to or that are specifically immunoreactive with an antibody or antisera generated against an immunogen comprising an amino acid sequence selected from one or more of the sequences given herein (e.g., SEQ ID NOS: 11-20, 27-32, and 39-44), etc. To eliminate cross-reactivity with other homologues, the antibody or antisera is subtracted with the HA and/or NA molecules found in public databases at the time of filing, e.g., the "control" polypeptide(s). Where the other control sequences correspond to a nucleic acid, a polypeptide encoded by the nucleic acid is generated and used for antibody/antisera subtraction purposes.

In one typical format, the immunoassay uses a polyclonal antiserum which was raised against one or more polypeptide comprising one or more of the sequences corresponding to the sequences herein (e.g., SEQ ID NOS: 11-20, 27-32, and 39-44), etc. or a substantial subsequence thereof (i.e., at least about 30% of the full length sequence provided). The set of potential polypeptide immunogens derived from the present sequences are collectively referred to below as "the immunogenic polypeptides." The resulting antisera is optionally selected to have low cross-reactivity against the control hemagglutinin and/or neuraminidase homologues and any such cross-reactivity is removed, e.g., by immunoabsorbtion,

with one or more of the control hemagglutinin and neuraminidase homologues, prior to use of the polyclonal antiserum in the immunoassay.

In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example, recombinant protein can be produced in a recombinant cell. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see, e.g., Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity). Additional references and discussion of antibodies is also found herein and can be applied here to defining polypeptides by immunoreactivity. Alternatively, one or more synthetic or recombinant polypeptide derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

Polyclonal sera are collected and titered against the immunogenic polypeptide in an immunoassay, for example, a solid phase immunoassay with one or more of the immunogenic proteins immobilized on a solid support. Polyclonal antisera with a titer of 10^6 or greater are selected, pooled and subtracted with the control hemagglutinin and/or neuraminidase polypeptide(s) to produce subtracted pooled titered polyclonal antisera.

The subtracted pooled titered polyclonal antisera are tested for cross reactivity against the control homologue(s) in a comparative immunoassay. In this comparative assay, discriminatory binding conditions are determined for the subtracted titered polyclonal antisera which result in at least about a 5-10 fold higher signal to noise ratio for binding of the titered polyclonal antisera to the immunogenic polypeptides as compared to binding to the control homologues. That is, the stringency of the binding reaction is adjusted by the addition of non-specific competitors such as albumin or non-fat dry milk, and/or by adjusting salt conditions, temperature, and/or the like. These binding conditions are used in subsequent assays for determining whether a test polypeptide (a polypeptide being compared to the immunogenic polypeptides and/or the control polypeptides) is specifically bound by the pooled subtracted polyclonal antisera. In particular, test polypeptides which show at least a 2-5× higher signal to noise ratio than the control receptor homologues under discriminatory binding conditions, and at least about a 1/2 signal to noise ratio as compared to the immunogenic polypeptide(s), shares substantial structural similarity with the immunogenic polypeptide as compared to the known receptor, etc., and is, therefore a polypeptide of the invention.

In another example, immunoassays in the competitive binding format are used for detection of a test polypeptide. For example, as noted, cross-reacting antibodies are removed from the pooled antisera mixture by immunoabsorbtion with the control polypeptides. The immunogenic polypeptide(s) are then immobilized to a solid support which is exposed to the subtracted pooled antisera. Test proteins are added to the assay to compete for binding to the pooled subtracted antisera. The ability of the test protein(s) to compete for binding to the pooled subtracted antisera as compared to the immobilized protein(s) is compared to the ability of the immunogenic polypeptide(s) added to the assay to compete for binding (the immunogenic polypeptides compete effectively with the immobilized immunogenic polypeptides for binding to the pooled antisera). The percent cross-reactivity for the test proteins is calculated, using standard calculations.

In a parallel assay, the ability of the control protein(s) to compete for binding to the pooled subtracted antisera is optionally determined as compared to the ability of the immunogenic polypeptide(s) to compete for binding to the antisera. Again, the percent cross-reactivity for the control polypeptide(s) is calculated, using standard calculations. Where the percent cross-reactivity is at least 5-10× as high for the test polypeptides as compared to the control polypeptide(s) and or where the binding of the test polypeptides is approximately in the range of the binding of the immunogenic polypeptides, the test polypeptides are said to specifically bind the pooled subtracted antisera.

In general, the immunoabsorbed and pooled antisera can be used in a competitive binding immunoassay as described herein to compare any test polypeptide to the immunogenic and/or control polypeptide(s). In order to make this comparison, the immunogenic, test and control polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the subtracted antisera to, e.g., an immobilized control, test or immunogenic protein is determined using standard techniques. If the amount of the test polypeptide required for binding in the competitive assay is less than twice the amount of the immunogenic polypeptide that is required, then the test polypeptide is said to specifically bind to an antibody generated to the immunogenic protein, provided the amount is at least about 5-10× as high as for the control polypeptide.

As an additional determination of specificity, the pooled antisera is optionally fully immunosorbed with the immunogenic polypeptide(s) (rather than the control polypeptide(s)) until little or no binding of the resulting immunogenic polypeptide subtracted pooled antisera to the immunogenic polypeptide(s) used in the immunosorbtion is detectable. This fully immunosorbed antisera is then tested for reactivity with the test polypeptide. If little or no reactivity is observed (i.e., no more than 2× the signal to noise ratio observed for binding of the fully immunosorbed antisera to the immunogenic polypeptide), then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

Nucleic Acid and Polypeptide Sequence Variants

As described herein, the invention provides for nucleic acid polynucleotide sequences and polypeptide amino acid sequences, e.g., hemagglutinin and neuraminidase sequences, and, e.g., compositions and methods comprising said sequences. Examples of said sequences are disclosed herein (e.g., SEQ ID NOS: 1-45). However, one of skill in the art will appreciate that the invention is not necessarily limited to those sequences disclosed herein and that the present invention also provides many related and unrelated sequences with the functions described herein, e.g., encoding a HA and/or a NA molecule.

One of skill will also appreciate that many variants of the disclosed sequences are included in the invention. For example, conservative variations of the disclosed sequences that yield a functionally identical sequence are included in the invention. Variants of the nucleic acid polynucleotide sequences, wherein the variants hybridize to at least one disclosed sequence, are considered to be included in the invention. Unique subsequences of the sequences disclosed herein, as determined by, e.g., standard sequence comparison techniques, are also included in the invention.

Silent Variations

Due to the degeneracy of the genetic code, any of a variety of nucleic acid sequences encoding polypeptides and/or viruses of the invention are optionally produced, some which

can bear lower levels of sequence identity to the HA and NA nucleic acid and polypeptide sequences herein. The following provides a typical codon table specifying the genetic code, found in many biology and biochemistry texts.

TABLE 1

Codon Table

Amino acids			Codon
Alanine	Ala	A	GCA GCC GCG GCU
Cysteine	Cys	C	UGC UGU
Aspartic acid	Asp	D	GAC GAU
Glutamic acid	Glu	E	GAA GAG
Phenylalanine	Phe	F	UUC UUU
Glycine	Gly	G	GGA GGC GGG GGU
Histidine	His	H	CAC CAU
Isoleucine	Ile	I	AUA AUC AUU
Lysine	Lys	K	AAA AAG
Leucine	Leu	L	UUA UUG CUA CUC CUG CUU
Methionine	Met	M	AUG
Asparagine	Asn	N	AAC AAU
Proline	Pro	P	CCA CCC CCG CCU
Glutamine	Gln	Q	CAA CAG
Arginine	Arg	R	AGA AGG CGA CGC CGG CGU
Serine	Ser	S	AGC AGU UCA UCC UCG UCU
Threonine	Thr	T	ACA ACC ACG ACU
Valine	Val	V	GUA GUC GUG GUU
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC UAU

The codon table shows that many amino acids are encoded by more than one codon. For example, the codons AGA, AGG, CGA, CGC, CGG, and CGU all encode the amino acid arginine. Thus, at every position in the nucleic acids of the invention where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described above without altering the encoded polypeptide. It is understood that U in an RNA sequence corresponds to T in a DNA sequence.

Such "silent variations" are one species of "conservatively modified variations," discussed below. One of skill will recognize that each codon in a nucleic acid (except ATG, which is ordinarily the only codon for methionine, and TTG, which is ordinarily the only codon for tryptophan) can be modified by standard techniques to encode a functionally identical polypeptide. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in any described sequence. The invention, therefore, explicitly provides each and every possible variation of a nucleic acid sequence encoding a polypeptide of the invention that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code (e.g., as set forth in Table 1, or as is commonly available in the art) as applied to the nucleic acid sequence encoding a hemagglutinin or a neuraminidase polypeptide of the invention. All such variations of every nucleic acid herein are specifically provided and described by consideration of the sequence in combination with the genetic code. One of skill is fully able to make these silent substitutions using the methods herein.

Conservative Variations

Owing to the degeneracy of the genetic code, "silent substitutions" (i.e., substitutions in a nucleic acid sequence which do not result in an alteration in an encoded polypeptide) are an implied feature of every nucleic acid sequence of the invention which encodes an amino acid. Similarly, "conservative amino acid substitutions," in one or a few amino acids in an amino acid sequence are substituted with different amino acids with highly similar properties, are also readily identified as being highly similar to a disclosed construct such as those herein. Such conservative variations of each disclosed sequence are a feature of the present invention.

"Conservative variations" of a particular nucleic acid sequence refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or, where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences, see, Table 2 below. One of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 4%, 3%, 2% or 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the deletion of an amino acid, addition of an amino acid, or substitution of an amino acid with a chemically similar amino acid. Thus, "conservative variations" of a listed polypeptide sequence of the present invention include substitutions of a small percentage, typically less than 5%, more typically less than 4%, 3%, 2% or 1%, of the amino acids of the polypeptide sequence, with a conservatively selected amino acid of the same conservative substitution group. Finally, the addition of sequences which do not alter the encoded activity of a nucleic acid molecule, such as the addition of a non-functional sequence, is a conservative variation of the basic nucleic acid.

TABLE 2

Conservative Substitution Groups

1	Alanine (A)	Serine (S)	Threonine (T)	
2	Aspartic acid (D)	Glutamic acid (E)		
3	Asparagine (N)	Glutamine (Q)		
4	Arginine (R)	Lysine (K)		
5	Isoleucine (I)	Leucine (L)	Methionine (M)	Valine (V)
6	Phenylalanine (F)	Tyrosine (Y)	Tryptophan (W)	

Unique Polypeptide and Polynucleotide Subsequences

In one aspect, the invention provides a nucleic acid which comprises a unique subsequence in a nucleic acid selected from the sequence of HA and NA molecules disclosed herein, e.g., SEQ ID NOS: 1-10, 21-26, 33-38, and 45. The unique subsequence is unique as compared to a nucleic acids corresponding to nucleic acids such as, e.g., those found in GenBank or other similar public databases at the time of filing. Alignment can be performed using, e.g., BLAST set to default parameters. Any unique subsequence is useful, e.g., as a probe to identify the nucleic acids of the invention. See, above.

Similarly, the invention includes a polypeptide which comprises a unique subsequence in a polypeptide selected from the sequence of HA and NA molecules disclosed herein, e.g., SEQ ID NOS: 11-20, 27-32, and 39-44. Here, the unique subsequence is unique as compared to a polypeptide corresponding to, e.g., the amino acid corresponding to polynucleotide sequences found in, e.g., GenBank or other similar public databases at the time of filing.

The invention also provides for target nucleic acids which hybridize under stringent conditions to a unique coding oligonucleotide which encodes a unique subsequence in a polypeptide selected from the sequences of HA and NA molecules of the invention wherein the unique subsequence is unique as compared to a polypeptide corresponding to any of the control polypeptides (sequences of, e.g., the nucleic acids corresponding to those found in, e.g., GenBank or other similar public databases at the time of filing). Unique sequences are determined as noted above.

Sequence Comparison, Identity, and Homology

The terms "identical" or percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (or other algorithms available to persons of skill) or by visual inspection.

The phrase "substantially identical," in the context of two nucleic acids or polypeptides (e.g., DNAs encoding a HA or NA molecule, or the amino acid sequence of a HA or NA molecule) refers to two or more sequences or subsequences that have at least about 90%, preferably 91%, most preferably 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. Such "substantially identical" sequences are typically considered to be "homologous," without reference to actual ancestry. Preferably, "substantial identity" exists over a region of the amino acid sequences that is at least about 200 residues in length, at least about 250 residues, at least about 300 residues, 350 residues, 400 residues, 425 residues, 450 residues, 475 residues, 480 residues, 490 residues, 495 residues, 499 residues, 500 residues, 502 residues, 559 residues, 565 residues, or 566 residues, or over the full length of the two sequences to be compared.

For sequence comparison and homology determination, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv Appl Math* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J Mol Biol* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc Natl Acad Sci USA* 85:2444 (1988), by computerized implementations of algorithms such as GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis., or by visual inspection (see generally, Ausubel et al., supra).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., *J Mol Biol* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (see, Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=−4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see, Henikoff & Henikoff (1989) *Proc Natl Acad Sci USA* 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc Natl Acad Sci USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Another example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle (1987) *J. Mol. Evol.* 35:351-360. The method used is similar to the method described by Higgins & Sharp (1989) *CABIOS*5:151-153. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster can then be aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences can be aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program can also be used to plot a dendogram or tree representation of clustering relationships. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison.

An additional example of an algorithm that is suitable for multiple DNA, or amino acid, sequence alignments is the CLUSTALW program (Thompson, J. D. et al. (1994) *Nucl. Acids. Res.* 22: 4673-4680). CLUSTALW performs multiple pairwise comparisons between groups of sequences and assembles them into a multiple alignment based on homology. Gap open and Gap extension penalties can be, e.g., 10 and 0.05 respectively. For amino acid alignments, the BLOSUM algorithm can be used as a protein weight matrix. See, e.g., Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919.

Digital Systems

The present invention provides digital systems, e.g., computers, computer readable media and integrated systems comprising character strings corresponding to the sequence information herein for the nucleic acids and isolated or recombinant polypeptides herein, including, e.g., the sequences shown herein, and the various silent substitutions and conservative substitutions thereof. Integrated systems can further include, e.g., gene synthesis equipment for making genes corresponding to the character strings.

Various methods known in the art can be used to detect homology or similarity between different character strings (see, above), or can be used to perform other desirable functions such as to control output files, provide the basis for making presentations of information including the sequences and the like. Examples include BLAST, discussed supra. Computer systems of the invention can include such programs, e.g., in conjunction with one or more data file or data base comprising a sequence as noted herein.

Thus, different types of homology and similarity of various stringency and length between various HA or NA sequences or fragments, etc. can be detected and recognized in the integrated systems herein. For example, many homology determination methods have been designed for comparative analysis of sequences of biopolymers, for spell-checking in word processing, and for data retrieval from various databases. With an understanding of double-helix pair-wise complement interactions among 4 principal nucleobases in natural polynucleotides, models that simulate annealing of complementary homologous polynucleotide strings can also be used as a foundation of sequence alignment or other operations typically performed on the character strings corresponding to the sequences herein (e.g., word-processing manipulations, construction of figures comprising sequence or subsequence character strings, output tables, etc.).

Thus, standard desktop applications such as word processing software (e.g., Microsoft Word™ or Corel WordPerfect™) and database software (e.g., spreadsheet software such as Microsoft Excel™, Corel Quattro Pro™, or database programs such as Microsoft Access™, Paradox™, GeneWorks™, or MacVector™ or other similar programs) can be adapted to the present invention by inputting a character string corresponding to one or more polynucleotides and polypeptides of the invention (either nucleic acids or proteins, or both). For example, a system of the invention can include the foregoing software having the appropriate character string information, e.g., used in conjunction with a user interface (e.g., a GUI in a standard operating system such as a Windows, Macintosh or LINUX system) to manipulate strings of characters corresponding to the sequences herein. As noted, specialized alignment programs such as BLAST can also be incorporated into the systems of the invention for alignment of nucleic acids or proteins (or corresponding character strings).

Systems in the present invention typically include a digital computer with data sets entered into the software system comprising any of the sequences herein. The computer can be, e.g., a PC (Intel x86 or Pentium chip-compatible DOS™, OS2™ WINDOWS™ WINDOWSNT™, WINDOWS95™, WINDOWS2000™, WINDOWS98™, LINUX based machine, a MACINTOSH™, Power PC, or a UNIX based (e.g., SUN™ work station) machine) or other commercially available computer that is known to one of skill. Software for aligning or otherwise manipulating sequences is available, or can easily be constructed by one of skill using a standard programming language such as Visualbasic, PERL, Fortran, Basic, Java, or the like.

Any controller or computer optionally includes a monitor which is often a cathode ray tube ("CRT") display, a flat panel display (e.g., active matrix liquid crystal display, liquid crystal display), or others. Computer circuitry is often placed in a box which includes numerous integrated circuit chips, such as a microprocessor, memory, interface circuits, and others. The box also optionally includes a hard disk drive, a floppy disk drive, a high capacity removable drive such as a writeable CD-ROM, and other common peripheral elements. Inputting devices such as a keyboard or mouse optionally provide for input from a user and for user selection of sequences to be compared or otherwise manipulated in the relevant computer system.

The computer typically includes appropriate software for receiving user instructions, either in the form of user input into a set parameter fields, e.g., in a GUI, or in the form of preprogrammed instructions, e.g., preprogrammed for a variety of different specific operations. The software then converts these instructions to appropriate language for instructing the operation, e.g., of appropriate mechanisms or transport controllers to carry out the desired operation. The software can also include output elements for controlling nucleic acid synthesis (e.g., based upon a sequence or an alignment of sequences herein), comparisons of samples for differential gene expression, or other operations.

Kits and Reagents

The present invention is optionally provided to a user as a kit. For example, a kit of the invention contains one or more nucleic acid, polypeptide, antibody, or cell line described herein (e.g., comprising, or with, a HA and/or NA molecule of the invention). The kit can contain a diagnostic nucleic acid or polypeptide, e.g., antibody, probe set, e.g., as a cDNA microarray packaged in a suitable container, or other nucleic acid such as one or more expression vector. The kit can also further comprise, one or more additional reagents, e.g., substrates, labels, primers, for labeling expression products, tubes and/or other accessories, reagents for collecting samples, buffers, hybridization chambers, cover slips, etc. The kit optionally further comprises an instruction set or user manual detailing preferred methods of using the kit components for discovery or application of diagnostic sets, etc.

When used according to the instructions, the kit can be used, e.g., for evaluating a disease state or condition, for evaluating effects of a pharmaceutical agent or other treatment intervention on progression of a disease state or condition in a cell or organism, or for use as a vaccine, etc.

In an additional aspect, the present invention provides system kits embodying the methods, composition, systems and apparatus herein. System kits of the invention optionally comprise one or more of the following: (1) an apparatus, system, system component or apparatus component; (2) instructions for practicing methods described herein, and/or for operating the apparatus or apparatus components herein and/or for using the compositions herein. In a further aspect, the present invention provides for the use of any apparatus, apparatus component, composition or kit herein, for the practice of any method or assay herein, and/or for the use of any apparatus or kit to practice any assay or method herein.

Additionally, the kits can include one or more translation system as noted above (e.g., a cell) with appropriate packaging material, containers for holding the components of the kit, instructional materials for practicing the methods herein and/or the like. Similarly, products of the translation systems (e.g., proteins such as HA and/or NA molecules) can be provided in kit form, e.g., with containers for holding the components of the kit, instructional materials for practicing the methods herein and/or the like.

To facilitate use of the methods and compositions of the invention, any of the vaccine components and/or compositions, e.g., reassorted virus in allantoic fluid, etc., and additional components, such as, buffer, cells, culture medium, useful for packaging and infection of influenza viruses for experimental or therapeutic vaccine purposes, can be packaged in the form of a kit. Typically, the kit contains, in addition to the above components, additional materials which can include, e.g., instructions for performing the methods of the invention, packaging material, and a container.

EXAMPLES

Example 1

Construction and Analysis of H5N1 ca Viruses and Vaccines

Various sequences herein comprising H5N1 HA/NA sequences were used to create influenza viruses and vaccines. The HA sequences in such vaccines were altered from wild-type by removal of the polybasic cleavage site within the HA. The HA/NA sequences were reassorted (in a 6:2 reassortment) with ca A/AA/6/60 (a ts, au, ca virus, see above).

Three strains of H5N1 influenza were used in this example: A/VN/1203/2004, A/HK/491/1997, and A/HK/213/2003. Such strains are also referred to within this example as the '97, '03, and '04 strains based on their year designations. The HA sequence homology of these three strains is 95-96%. FIG. **1** illustrates modification of the polybasic cleavage site of an exemplary HA sequence, the '04 HA sequences, used to construct the viruses/vaccines. As stated previously, various embodiments of the invention comprise sequences which have differing regions of the polybasic cleavage site removed. See above.

As stated, the modified H5N1 sequences (i.e., the modified '97, '03, and '04 genes) were used to construct 6:2 reassortant viruses with ca A/AA/6/60. It will be appreciated, and is pointed out elsewhere herein, that other desirable backbones could also have been used (e.g., PR8, etc.).

In the 6:2 reassortants of this example, the HA and NA gene sequences were derived from one or more wild type parent virus, i.e., the HA and NA gene sequences of the '03 virus were derived from A/HK/213/2003, the HA and NA gene sequences of the '04 virus were derived from A/VN/1203/2004, and the HA gene sequence of the '97 virus was derived from A/HK/491/1997 while the NA gene sequence was derived from A/HK/486/1997. The remaining genes of the 6:2 reassortants were characterized by sequence analysis as derived from the A/AA/6/60 ca parent virus. The reassorted viruses replicated to 8.0-8.5 $\log_{10}TCID_{50}$ in eggs. However, it will be appreciated that other embodiments wherein the $\log_{10}TCID_{50}$ comprises from about 7.0 to about 9.0, from about 7.5-8.5, or from about 8.0-8.5 are also within the scope of the invention. The cleavability of the modified HA in the constructed viruses by endogenous proteases was restricted in vitro and the viruses were dependent on trypsin (e.g., from about 0.1 ug/ml to about 1.0 ug/ml) for growth. The constructed viruses were temperature sensitive as assayed by an in vitro assay.

The H5N1 ca reassortant viruses (having the modified '97, '03, or '04 HA genes) were not lethal for chickens. For example, when 4-week-old SPF white Plymouth Rock chickens were inoculated intravenously with a 1:10 dilution of stock virus ($10^{8-8.75}$ $TCID_{50}$/ml) and observed for 10 days, it was observed that 8 out of 8 chickens died within 1-2 days when wild-type '97, '03, and '04 H5N1 were used, while 0 of 8 chickens died when the H5N1 ca reassortant viruses were used. As can be seen in FIG. **2**, the intranasally administered H5N1 ca reassortant viruses did not replicate in chickens.

The H5N1/AA ca reassortants were also not lethal for mice. See FIG. **3**, which also shows the $TCID_{50}$ for the H5N1 wild-type strains. FIG. **4** shows that the 1997 and 2004 H5N1 ca reassortant viruses were restricted in replication in mice. FIG. **5**, shows that the H5N1 ca reassorted viruses are restricted in replication in lungs of mice.

A comparison of the serum HAI antibody titers elicited in mice following a single intranasal dose of vaccine (2003 ca as compared against 2003 wild-type), is shown in FIG. **6**. FIGS. **7** and **15** show similar measurements, but using serum neutralizing antibody titers.

FIG. **8** displays that the H5N1 ca reassortant viruses protect mice from lethal challenge with 50, 500, or 5,000 LD_{50} of wild-type H5N1 virus. FIG. **9** shows the efficacy of protection from pulmonary replication of homologous and heterologous H5N1 challenge viruses in mice. As can be seen, the ca reassortants replicated less well than the wild-type viruses did. FIG. **10** shows related data using upper respiratory tracts of mice. Those of skill in the art will be familiar with homologous and heterologous challenges (e.g., testing whether 2003 vaccine protects against a 2003 wild-type challenge (homologous) or whether a 2003 vaccine protects against a 1997 wild-type challenge (heterologous), etc.).

FIG. **11** shows efficacy of protection conferred by 2004 H5N1 ca vaccine against high dose (10^5TCID_{50}) challenge with homologous or heterologous H5N1 wild-type viruses in mice. FIG. **12** shows efficacy of protection conferred by 1997 and 2003 H5N1 ca vaccines against high dose (10^5TCID_{50}) challenge with homologous or heterologous H5N1 wild-type viruses in mice. FIG. **13** shows efficacy of protection conferred by 2004 H5N1 ca vaccine against low or high doses of homologous H5N1 wild-type virus challenge in mice. FIGS. **11-13** demonstrate that the tested vaccines could protect against other related viruses.

In healthy human adults nasal spray administration the '04 vaccine was well tolerated and its replication was highly restricted. See FIG. **27** for replication restriction of the vaccine in healthy adults. HI antibody responses to $10^{6.7}$ $TCID_{50}$ of the '04 vaccine were also observed in some of the healthy adults. See FIG. 28.

The current example demonstrates several points concerning exemplary H5N1 ca reassortant viruses/vaccines of the invention. The modified ca reassortant '97, '03, and '04 viruses were shown to have in vitro is phenotype, loss of pathogenicity in chickens and attenuation in mice. It is expected that attenuation is also present in ferrets. Efficacy of protection and cross-protection against lethal challenge and systemic spread with wild-type viruses in mice was also shown. Efficacy of protection and cross-protections against replication of wild-type challenge viruses in the respiratory tract of mice is also expected.

It is contemplated to use these (and similar) viruses/vaccines to determine whether immunogenicity and efficacy is improved following 2 doses of vaccine; to assess immunogenicity in non-human primates; to assess attenuation and vaccine efficacy in ferrets; to determine the contribution of humoral and cellular immunity to observed efficacy of the produced vaccines in mice; to determine which residues of the 2003 HA contribute to enhanced immunogenicity and introduce them into 1997 and 2004 HAs; and to determine the effects of deleting the multibasic amino acid cleavage site and of the gene constellation.

Example 2

Construction and Analysis of H6 ca Viruses and Vaccines

A set of three recombinant influenza viruses and vaccines comprising H6 HA sequences were prepared: (a) A/Duck, which comprised the H6 HA and N9 NA of A/Duck77; (b) A/Teal, which comprised the H6 HA and N1 NA of A/Tea197; and (c) A/Mallard, which comprised the H6 HA and N2 NA of A/Mallard85. The six internal genome segments of each recombinant virus were those of ca A/AA/6/60.

Each of the A/Duck, A/Teal, and A/Mallard recombinant viruses was attenuated in nasal turbinates and lungs of ferrets. Ferrets were intranasally inoculated with 10^7 $TCID_{50}$ recombinant (ca; see paragraph immediately above) or wild-type (wt) H6 influenza virus. Nasal turbinate and lung tissue was harvested from the ferrets three days post-infection for examination. FIG. **16** shows that the nasal turbinate and lung tissue of ferrets inoculated with recombinant virus (ca) exhibited lower virus titers than did the nasal turbinate and lung tissue of ferrets inoculated with the respective counterpart wt virus.

Each of the A/Duck, A/Teal, and A/Mallard recombinant (ca) viruses was also immunogenic in the ferrets. See FIG. **17**.

FIG. **18** shows the efficacy of protection conferred by the A/Duck, A/Teal, and A/Mallard vaccines. Ferrets were vaccinated with a single dose of 7 $\log_{10}$ PFU recombinant A/Duck, A/Teal, or A/Mallard vaccine. The ferrets were then challenged with 7 $\log_{10}$ PFU wt A/Duck, A/Teal, or A/Mallard virus. Three days post challenge lungs and nasal turbinates of the ferrets were harvested and virus titer in the tissues was determined. FIG. **18** shows efficacy of protection conferred by the recombinant (ca) H6 vaccines against homologous and heterologous wild-type H6 viruses in ferrets.

Example 3

Construction and Analysis of an H7N3, BC 04 ca, Virus and Vaccine

A further recombinant influenza virus and vaccine was prepared using the HA H7 and NA N3 sequences of A/ck/BC/CN-6/04 (BC 04 ca). These HA and NA sequences were combined with the six internal genome segments of ca A/AA/6/60.

The BC 04 ca vaccine was attenuated in the ferrets. Ferrets were intranasally inoculated with 10^7 $TCID_{50}$ vaccine in 0.5 mL. Three days following inoculation, ferret nasal turbinates, lungs, brain, and olfactory bulb were harvested. Virus titer in each of these tissues was diminished in ferrets inoculated with the vaccine virus relative to ferrets inoculated with wt viruses A/BC/CN-6/04 or A/BC/CN-7/04. See FIG. **19**.

The BC 04 ca vaccine was immunogenic in mice. In mice receiving the BC 04 ca vaccine, neutralizing antibodies were detected at 4 weeks and these titers rose over 8 weeks. A second dose of vaccine boosted antibody titer but final titer achieved was similar to that following a single dose. See FIG. **20**.

FIGS. **21** and **22** show the efficacy of protection conferred by the BC 04 ca vaccine against both homologous and heterologous H7 wt viruses. For FIG. **21**, mice were intranasally inoculated with 1 dose vaccine four weeks before challenge, 1 dose vaccine 8 weeks before challenge, or 2 doses vaccine (administered 4 weeks apart) before lethal challenge with 50 LD_{50} homologous (A/ck/BC/CN-7/04) and heterologous (A/NL/219/03 or A/tk/Eng/63) H7 wt viruses. Weight change of the mice following lethal challenged was monitored each day for fourteen days, to monitor morbidity associated with the wt influenza virus challenge.

For each of the mice lethally challenged with the homologous A/ck/BC/CN-7/04 virus little or no weight change was observed regardless of whether 1 dose of vaccine was administered 4 weeks prior to challenge (a), 1 dose of vaccine was administered 8 weeks prior to challenge (b) or 2 doses of vaccine were administered prior to challenge (c). Likewise, little to no weight loss occurred following challenge of the mice with either heterologous influenza virus, A/NL/2109/03 (d, e, f) or A/tk/Eng/63 (g, h, i). Again, the lack of weight loss was observed regardless of whether 1 dose of vaccine was administered 4 weeks prior to challenge (d or g), 1 dose of vaccine was administered 8 weeks prior to challenge (e, or h), or 2 doses of vaccine were administered prior to challenge (f or i).

FIG. **22** provides further evidence of the efficacy of the H7N3 BC 04 ca vaccine. In both nasal turbinates (a) and lungs (b) of mice receiving the H7N3 BC 04 ca vaccine, protection was observed against challenge using ck/BC/CN-6/04 (H7N3), ck/BC/CN-7/04 (H7N3), NL/219/03 (H7N7), tk/Eng/63 (H7N3), tk/UT/95 (H7N3), and tk/VA/02 (H7N2) viruses.

Example 4

Construction and Analysis of an H9N2 G9/AA ca, Virus and Vaccine

A further recombinant influenza virus and vaccine was prepared using the HA H9 and NA N2 sequences of A/ck/Hong Kong/G9/97 (G9/AA ca). These HA and NA sequences were combined with the six internal genome segments of ca A/AA/6/60.

The H9N2 G9/AA ca vaccine was attenuated in the ferrets. See FIG. **23**, which shows reduced virus titers in nasal turbinates (a) and lungs (b) of ferrets following administration of the H9N2 G9/AA ca virus relative to the H9N2 G9 wt virus.

FIG. **24** provides evidence of the efficacy of the H9N2 G9 ca vaccine in mice. In the mice receiving the H9N2 G9 ca vaccine, protection was observed against challenge using H9N2 G9 wt and A/HK/1073/99 viruses.

The H9N2 G9/AA ca vaccine was also well tolerated in healthy adults in a clinical trial setting. Healthy adults were administered the H9N2 G9/AA ca vaccine by nose drop. In the healthy adults, the H9N2 G9/AA ca vaccine was highly restricted in replication. See FIG. **25**. Furthermore, administration of $10^{7\circ}$ $TCID_{50}$ of H9N2 G9/AA ca vaccine induced ≧4-fold increases in HI titer in 92% of healthy volunteers. See FIG. **26**.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and apparatus described above may be used in various combinations. All publications, patents, patent applications, or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, or other document were individually indicated to be incorporated by reference for all purposes. In particular, U.S. provisional application Nos. 60/821,832 filed Aug. 9, 2006 and 60/942,804, filed Jun. 8, 2007, are incorporated herein in their entirety for all purposes.

SPECIFIC EMBODIMENTS

Additional embodiments of the present invention are presented in Table 3 and 4.

TABLE 3

	Specific embodiments
1	An isolated polypeptide, wherein said polypeptide is selected from the group consisting of: a) a polypeptide encoded by a polynucleotide sequence as shown in any one of SEQ ID NOS: 21-26 or 33-38 or 45; b) a polypeptide as shown in any one of SEQ ID NOS: 27-32 or 39-44; c) the mature form of the polypeptide as shown in any one of SEQ ID NOS: 27-32 or 39-44; d) a polypeptide encoded by a polynucleotide sequence which hybridizes under highly stringent conditions to a polynucleotide sequence encoding (a) (b) or (c); and e) a polypeptide having at least 90% sequence identity to the polypeptide of (b).
2	An immunogenic composition comprising an immunologically effective amount of at least one polypeptide of embodiment 1.
3	An isolated antibody that specifically binds the polypeptide of embodiment 1.
4	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the polypeptide of embodiment 1 in a physiologically acceptable carrier.
5	A recombinant influenza virus comprising the polypeptide of embodiment 1.
6	An immunogenic composition comprising an immunologically effective amount of the recombinant influenza virus of embodiment 5.
7	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the recombinant influenza virus of embodiment 5 in a physiologically acceptable carrier.
8	An isolated nucleic acid, wherein said nucleic acid is selected from the group consisting of: a) a polynucleotide sequence as shown in any one of SEQ ID NOS: 21-26 or 33-38 or 45, or a complementary sequence thereof; b) a polynucleotide sequence encoding a polypeptide as shown in any one of SEQ ID NOS: 27-32 or 39-44, or a complementary polynucleotide sequence thereof; c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a); and d) a polynucleotide sequence having at least 98% sequence identity to the polynucleotide sequence of (a).
9	An immunogenic composition comprising at least one of the nucleic acids of embodiment 8.
10	A cell comprising at least one nucleic acid of embodiment 8.
11	A vector comprising the nucleic acid of embodiment 8.
12	The vector of embodiment 12, wherein the vector is a plasmid, a cosmid, a phage, a virus, or a fragment of a virus.
13	The vector of embodiment 12, wherein the vector is an expression vector.
14	A cell comprising the vector of embodiment 13.
15	An influenza virus comprising one or more nucleic acids of embodiment 8.
16	The virus of embodiment 15, wherein the virus is a reassortment virus.
17	A 6:2 reassortment influenza virus, wherein said virus comprises 6 gene encoding regions from A/Ann Arbor/6/60 and 2 gene encoding regions that encode polypeptides selected from the group consisting of: the polypeptides of SEQ ID NOS: 27-32, and 39-44.
18	A method of producing a recombinant influenza virus, the method comprising: culturing the cell of embodiment 14 in a suitable culture medium under conditions permitting expression of nucleic acid; and, isolating the recombinant influenza virus from a cell population comprising said cell or the medium.
19	An immunogenic composition comprising an immunologically effective amount of the recombinant influenza virus of embodiment 17.
20	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the recombinant influenza virus of embodiment 17 in a physiologically effective carrier.
21	A method of producing an isolated or recombinant polypeptide, the method comprising: culturing the host cell of embodiment 10 in a suitable culture medium under conditions permitting expression of said nucleic acid; and, isolating the polypeptide from one or more of the host cells or the medium.
22	A method of prophylactic or therapeutic treatment of a viral infection in a subject, the method comprising: administering to the subject, a virus of embodiment 17 in an amount effective to produce an immunogenic response against the viral infection.
23	The method of embodiment 22, wherein the subject is a human.
24	The immunogenic composition of embodiment 19, wherein the hemagglutinin comprises a modified polybasic cleavage site.
25	A live attenuated influenza vaccine comprising the composition of embodiment 19.
26	A split virus or killed virus vaccine comprising the composition of embodiment 19.
27	A live attenuated influenza vaccine comprising the composition of embodiment 24.
28	A split virus or killed virus vaccine comprising the composition of embodiment 24.
29	A method for producing influenza viruses in cell culture, the method comprising: i) introducing into a population of host cells, which population of host cells is capable of

TABLE 3-continued

	Specific embodiments
	supporting replication of influenza virus, a plurality of vectors comprising nucleic acid encoding at least 6 internal genome segments of a first influenza strain, wherein the first influenza strain is A/Ann Arbor/6/60; and, at least one genome segment encoding an immunogenic influenza surface antigen of a second influenza strain, wherein said second strain is a pandemic influenza strain, ii) culturing the population of host cells at a temperature less than or equal to 35° C.; and, iii) recovering a plurality of influenza viruses.
30	The method of embodiment 29, wherein the plurality of vectors comprise at least one isolated nucleic acid, wherein said nucleic acid is selected from the group consisting of: a) a polynucleotide sequence of one of SEQ ID NOS: 21-26 or 33-38, or 45, or a complementary sequence thereof; b) a polynucleotide sequence encoding a polypeptide of one of SEQ ID NOS: 27-32 or 39-44, or a complementary polynucleotide sequence thereof; c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a); and d) a polynucleotide sequence having at least 98% sequence identity to the polynucleotide sequence of (a).
31	An immunogenic composition comprising an immunologically effective amount of the influenza virus of embodiment 29.
32	An immunogenic composition comprising an immunologically effective amount of the influenza virus of embodiment 30.
33	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the influenza virus of embodiment 29 in a physiologically effective carrier.
34	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the influenza virus of embodiment 30 in a physiologically effective carrier.
35	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual the immunogenic composition of embodiment 31.
36	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual the immunogenic composition of embodiment 32.
37	A live attenuated influenza vaccine comprising the immunogenic composition of embodiment 31.
38	A split virus or killed virus vaccine comprising the immunogenic composition of embodiment 32.

TABLE 4

	Specific embodiments.
1	An isolated polypeptide, wherein said polypeptide is selected from the group consisting of: a) a polypeptide comprising the amino acid sequence encoded by the nucleotide sequence as shown in any one of SEQ ID NOS: 21-26 or 33-38 or 45; b) a polypeptide comprising the amino acid sequence as shown in any one of SEQ ID NOS: 27-32 or 39-44; c) the mature form of a polypeptide comprising the amino acid sequence as shown in any one of SEQ ID NOS: 27-32 or 39-44; d) a polypeptide comprising an amino acid sequence encoded by a polynucleotide which hybridizes under highly stringent conditions to a polynucleotide comprising a nucleotide sequence encoding (a) (b) or (c); and e) a polypeptide comprising an amino acid sequence having at least 90% sequence identity to the polypeptide of (b).
2	An immunogenic composition comprising an immunologically effective amount of at least one polypeptide of embodiment 1.
3	An isolated antibody that specifically binds the polypeptide of embodiment 1.
4	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the polypeptide of embodiment 1 in a physiologically acceptable carrier.
5	A recombinant influenza virus comprising the polypeptide of embodiment 1.
6	An immunogenic composition comprising an immunologically effective amount of the recombinant influenza virus of embodiment 5.
7	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the recombinant influenza virus of embodiment 5 in a physiologically acceptable carrier.
8	An isolated polynucleotide, wherein said polynucleotide is selected from the group consisting of: a) a polynucleotide comprising the nucleotide sequence as shown in any one of SEQ ID NOS: 21-26 or 33-38 or 45, or a complementary sequence thereof;

TABLE 4-continued

	Specific embodiments.
	b) a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising the amino acid sequence as shown in any one of SEQ ID NOS: 27-32 or 39-44, or a complementary nucleotide sequence thereof; c) a polynucleotide which hybridizes under highly stringent conditions over substantially the entire length of the polynucleotide of (a); and d) a polynucleotide comprising a nucleotide sequence having at least 98% sequence identity to the polynucleotide of (a).
9	An immunogenic composition comprising at least one polynucleotide of embodiment 8.
10	A cell comprising at least one polynucleotide of embodiment 8.
11	A vector comprising the polynucleotide of embodiment 8.
12	The vector of embodiment 11, wherein the vector is a plasmid, a cosmid, a phage, a virus, or a fragment of a virus.
13	The vector of embodiment 12, wherein the vector is an expression vector.
14	A cell comprising the vector of embodiment 13.
15	An influenza virus comprising one or more polynucleotides of embodiment 8.
16	The virus of embodiment 15, wherein the virus is a reassortant virus.
17	A 6:2 reassortant influenza virus, wherein said virus comprises 6 internal genome segments from A/Ann Arbor/6/60 and 2 genome segments that encode an HA and/or a NA polypeptide selected from the group consisting of: the polypeptides of SEQ ID NOS: 27-32, and 39-44.
18	A method of producing a reassortant influenza virus, the method comprising: culturing the cell of embodiment 14 in a suitable culture medium under conditions permitting expression of said polynucleotide; and, isolating the reassortant influenza virus from a cell population comprising said cell or the medium.
19	An immunogenic composition comprising an immunologically effective amount of the reassortant influenza virus of embodiment 17.
20	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the reassortant influenza virus of embodiment 17 in a physiologically effective carrier.
21	A method of producing an isolated or recombinant polypeptide, the method comprising: culturing the cell of embodiment 10 in a suitable culture medium under conditions permitting expression of said polynucleotide; and, isolating the polypeptide from the cell or the medium.
22	A method of prophylactic or therapeutic treatment of a viral infection in a subject, the method comprising: administering to the subject, the virus of embodiment 17 in an amount effective to produce an immunogenic response against the viral infection.
23	The method of embodiment 22, wherein the subject is a human.
24	The immunogenic composition of embodiment 19, wherein the hemagglutinin comprises a modified polybasic cleavage site.
25	A live attenuated influenza vaccine comprising the composition of embodiment 19.
26	A split virus or killed virus vaccine comprising the composition of embodiment 19.
27	A live attenuated influenza vaccine comprising the composition of embodiment 24.
28	A split virus or killed virus vaccine comprising the composition of embodiment 24.
29	A method for producing an influenza virus in cell culture, the method comprising: i) introducing into a population of host cells, which population of host cells is capable of supporting replication of influenza virus, a plurality of vectors comprising nucleotide sequences corresponding to at least 6 internal genome segments of A/Ann Arbor/6/60; and, at least one genome segment comprising a polynucleotide encoding an HA and/or a NA polypeptide selected from the group consisting of: the polypeptides of SEQ ID NOS: 27-32, and 39-44, ii) culturing the population of host cells at a temperature less than or equal to 35° C.; and, iii) recovering an influenza virus.
30	The method of embodiment 29, wherein the polynucleotide encoding the HA and/or NA polypeptide is selected from the group consisting of: a) a polynucleotide comprising the nucleotide sequence of any one of SEQ ID NOS: 21, 23-26 or 33-38, or 45, or a complementary nucleotide sequence thereof; b) a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising the amino acid sequence as shown in any one of SEQ ID NOS: 27-32 or 39-44, or a complementary nucleotide sequence thereof; c) a polynucleotide which hybridizes under highly stringent conditions over substantially the entire length of the polynucleotide of (a); and d) a polynucleotide comprising a nucleotide sequence having at least 98% sequence identity to the polynucleotide of (a).
31	An immunogenic composition comprising an immunologically effective amount of the influenza virus produced by the method of embodiment 29.
32	An immunogenic composition comprising an immunologically effective amount of the influenza virus produced by the method of embodiment 30.
33	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the influenza virus produced by the method of embodiment 29 in a physiologically effective carrier.
34	A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the influenza virus produced by the method of embodiment 30 in a physiologically effective carrier.

TABLE 4-continued

Specific embodiments.
35 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual the immunogenic composition of embodiment 31.
36 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual the immunogenic composition of embodiment 32.
37 A live attenuated influenza vaccine comprising the immunogenic composition of embodiment 31.
38 A split virus or killed virus vaccine comprising the immunogenic composition of embodiment 32.
39 A 6:2 reassortant influenza virus, wherein said virus comprises 6 internal genome segments from one or more donor viruses other than A/Ann Arbor/6/60 and 2 genome segments that encode an HA and/or a NA polypeptide selected from the group consisting of: the polypeptides of SEQ ID NOS: 27-32, and 39-44.
40 The 6:2 reassortant influenza virus of embodiment 39, wherein said donor virus comprises one or more of the following phenotypes: temperature-sensitive, cold-adaped, or attenuated.
41 The 6:2 reassortant influenza virus of embodiment 39, wherein said donor virus is PR8.
42 The 6:2 reassortant influenza virus of embodiment 39, wherein said donor virus is A/Leningrad/17.
43 An immunogenic composition comprising an immunologically effective amount of the reassortant influenza virus of embodiment 39.
44 An immunogenic composition comprising an immunologically effective amount of the reassortant influenza virus of embodiment 40.
45 An immunogenic composition comprising an immunologically effective amount of the reassortant influenza virus of embodiment 41.
46 An immunogenic composition comprising an immunologically effective amount of the reassortant influenza virus of embodiment 42.
47 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the reassortant influenza virus of embodiment 39 in a physiologically effective carrier.
48 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the reassortant influenza virus of embodiment 40 in a physiologically effective carrier.
49 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the reassortant influenza virus of embodiment 41 in a physiologically effective carrier.
50 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the reassortant influenza virus of embodiment 42 in a physiologically effective carrier.
51 A method of prophylactic or therapeutic treatment of a viral infection in a subject, the method comprising: administering to the subject, the virus of embodiment 39 in an amount effective to produce an immunogenic response against the viral infection.
52 A method of prophylactic or therapeutic treatment of a viral infection in a subject, the method comprising: administering to the subject, the virus of embodiment 41 in an amount effective to produce an immunogenic response against the viral infection.
53 A method of prophylactic or therapeutic treatment of a viral infection in a subject, the method comprising: administering to the subject, the virus of embodiment 42 in an amount effective to produce an immunogenic response against the viral infection.
54 The method of embodiment 51, wherein said virus is killed or inactivated.
55 The method of embodiment 52, wherein said virus is killed or inactivated.
56 The method of embodiment 53, wherein said virus is killed or inactivated.
57 The immunogenic composition of embodiment 43, wherein the hemagglutinin comprises a modified polybasic cleavage site.
58 The immunogenic composition of embodiment 44, wherein the hemagglutinin comprises a modified polybasic cleavage site.
59 The immunogenic composition of embodiment 45, wherein the hemagglutinin comprises a modified polybasic cleavage site
60 The immunogenic composition of embodiment 46, wherein the hemagglutinin comprises a modified polybasic cleavage site.
61 The method of embodiment 47, wherein the subject is a human.
62 The method of embodiment 48, wherein the subject is a human.
63 The method of embodiment 49, wherein the subject is a human.
64 A live attenuated influenza vaccine comprising the composition of embodiment 45.
65 A live attenuated influenza vaccine comprising the composition of embodiment 46.
66 A method for producing an influenza virus in cell culture, the method comprising: i) introducing into a population of host cells, which population of host cells is capable of supporting replication of influenza virus, a plurality of vectors comprising nucleotide sequences corresponding to: (a) at least 6 internal genome segments of a first influenza strain, wherein the first influenza strain is not A/Ann Arbor/6/60; and, at least one genome segment encoding an HA or an NA polypeptide selected from the group consisting of: the polypeptides of SEQ ID NOS: 27-32, and 39-44; or

TABLE 4-continued

Specific embodiments.
(b) at least 6 internal genome segments of a first influenza strain, wherein the first influenza strain is not A/Ann Arbor/6/60 and which influenza strain comprises one or more phenotypic attributes selected from the group consisting of: attenuated, cold adapted and temperature sensitive; and, at least one genome segment encoding an HA or an NA polypeptide selected from the group consisting of: the polypeptides of SEQ ID NOS: 27-32, and 39-44, ii) culturing the population of host cells at a temperature less than or equal to 35° C.; and, iii) recovering an influenza virus.
67 An immunogenic composition comprising an immunologically effective amount of the influenza virus produced by the method of embodiment 66.
68 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the influenza virus produced by the method of embodiment 66 in a physiologically effective carrier.
69 A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual the immunogenic composition of embodiment 67.
70 A live attenuated influenza vaccine comprising the immunogenic composition of embodiment 67.
71 A split virus or killed virus vaccine comprising the immunogenic composition of embodiment 67.

SEQUENCE LISTING

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gggataattg gcatggctcg aaccgaccat gggtgtcttt caatcagaat ctggagtatc 900

aaataggata tatatgcagt ggggttttcg gagacagtcc acgccccaat gatgggacag 960

gcagttgtgg tccagtgtct cttaacggag cgtatggagt aaaagggttt tcatttaaat 1020

acggcaatgg tgtttggatc gggagaacca aaagcactag ttccaggagc ggttttgaaa 1080

tgatttggga tccaaatggg tggaccgaaa cagacagtag cttctcgttg aagcaagaca 1140

tcatagcgat aactgattgg tcaggataca gcgggagttt tattcaacat ccagaactga 1200

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caggattaaa ttgcatgaga ccttgcttct gggttgaact aatcagaggg aggcccaaag 1260

agaaaacaat ctggactagt gggagcagta tatctttctg tggtgtaaat agtgacactg 1320

tgggttggtc ttggccagac ggtgctgagt tgccatacac cattgacaag tagtttgttc 1380

aaaaaactcc ttgtttctac t 1401

<210> SEQ ID NO 7
<211> LENGTH: 1767
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 7

agcaaaagca ggggtataat ctgtcaaaat ggagaaaata gtgcttcttc ttgcaacagt 60

cagccttgtt aaaagtgacc agatttgcat tggttaccat gcaaacaact cgacagagca 120

agttgacaca ataatggaaa agaatgttac tgttacacat gcccaagaca tactggaaag 180

gacacacaac gggaagctct gcgatctaaa tggagtgaag cctctgattt tgagggattg 240

tagtgtagct ggatggctcc tcggaaaccc tatgtgtgac gaattcatca atgtgccgga 300

atggtcttac atagtggaga aggccagtcc agccaatgac ctctgttatc cagggaattt 360

caacgactat gaagaactga aacacctatt gagcagaata aaccattttg agaaaattca 420

gataatcccc aaaagttctt ggtccaatca tgatgcctca tcaggggtga gctcagcatg 480

tccatacctt gggaggtcct ccttttttcag aaatgtggta tggcttatca aaaagaacag 540

tagctaccca acaataaaga ggagctacaa taataccaac caagaagatc ttttggtact 600

gtgggggatt caccatccta atgatgcggc agagcagaca aggctctatc aaaacccaac 660

cacctacatt tccgttggaa catcaacact gaaccagaga ttggtttcag aaatagctac 720

tagacccaaa gtaaacgggc aaagtggaag aatggagttc ttctggacaa ttttaaagcc 780

gaatgatgcc atcaatttcg agagtaatgg aaatttcatt gctccagaat atgcatacaa 840

aattgtcaag aaaggggact caacaattat gaaaagtgaa ttggaatatg gtaactgcaa 900

caccaagtgt caaactccaa tgggggcaat aaactctagt atgccattcc acaacataca 960

ccccctcacc atcggggaat gccccaaata tgtgaaatca aacagattag tccttgcaac 1020

tggactcaga aataccctc aacgagagac gcgaggacta tttggagcta tagcaggttt 1080

tatagaggga ggatggcagg gaatggtaga tggttggtat gggtaccacc atagcaatga 1140

gcaggggagt ggatacgctg cagaccaaga atccacacaa aaggcaatag atggagtcac 1200

caataaggtc aactcgatca ttaacaaaat gaacactcag tttgaggccg ttggaaggga 1260

atttaataac ttggaaagga ggatagagaa tttaaacaag aaaatggaag acggattcct 1320

agatgtctgg acttacaatg ccgaacttct ggttctcatg gaaaatgaga gaactctaga 1380

ctttcatgac tcaaatgtca agaaccttta cgacaaggtc cgactacagc ttagggataa 1440

tgcaaaggag ctgggtaatg gttgtttcga attctatcac aaatgtgata acgaatgtat 1500

ggaaagtgta aaaaacggaa cgtatgacta cccgcagtat tcagaagaag caagactaaa 1560

cagagaggaa ataagtggag taaaattgga atcaatggga acttaccaaa tactgtcaat 1620

ttattcaaca gtggcgagtt ccctagcact ggcaatcatg gtagctggtc tatctttatg 1680

gatgtgctcc aatggatcgt tacaatgcag aatttgcatt taaatttgtg agttcagatt 1740

gtagttaaaa acacccttgt ttctact 1767

<210> SEQ ID NO 8
<211> LENGTH: 1401
<212> TYPE: DNA

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<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 8

agcaaaagca ggagtttaaa atgaatccaa atcagaagat aataaccatt ggatcaatct 60

gcatggtagt tgggataatc agcttgatgt tacaaattgg aaacacaata tcagtatggg 120

tcagccacat aattaaaact tggcacccaa accagcctga accatgcaac caaagcatca 180

atttttacac tgagcaggct gcagcttcag tgacattagc gggcaattcc tctctctgcc 240

ctattagtgg atgggctata tacagcaagg acaatagtat aagaattggt tccaaagggg 300

atgtgtttgt tataagagaa ccattcatct catgctccca tttggaatgc agaacctttt 360

tcttgaccca aggagcccta ttgaatgaca agcattctaa tgggaccgtc aaagacagga 420

gcccctatag aactttaatg agctgtcctg ttggtgaggc cccttcccca tacaactcaa 480

ggtttgagtc tgttgcttgg tcagcaagtg cttgccatga tggcattagt tggctaacaa 540

ttggaatttc cggtccggat aatggggctg tggctgtgtt gaaatacaat ggcataataa 600

cagacaccat caagagttgg aggaacaaca cactgaggac gcaagagtct gaatgtgcat 660

gtgtgaatgg ttcttgtttt actgtaatga cagatggacc gagtaatgaa caggcctcat 720

acaagatttt caagatagaa aaggggaggg tagtcaaatc agttgagttg aacgccccta 780

attatcatta cgaggaatgc tcctgttatc ctgatgctgg cgaaatcaca tgtgtgtgca 840

gggataattg gcatggctcg aaccgaccat gggtgtcttt caatcagaat ctggagtatc 900

aaataggata tatatgcagt ggggttttcg gagacagtcc acgccccaat gatgggacag 960

gcagttgtgg tccagtgtct cttaacggag cgtatggagt aaaagggttt tcatttaaat 1020

acggcaatgg tgtttggatc gggagaacca aaagcactag ttccaggagc ggttttgaaa 1080

tgatttggga tccaaatggg tggaccgaaa cagacagtag cttctcgttg aagcaagaca 1140

tcatagcgat aactgattgg tcaggataca gcgggagttt tattcaacat ccagaactga 1200

caggattaaa ttgcatgaga ccttgcttct gggttgaact aatcagaggg aggcccaaag 1260

agaaaacaat ctggactagt gggagcagta tatctttctg tggtgtaaat agtgacactg 1320

tgggttggtc ttggccagac ggtgctgagt tgccatacac cattgacaag tagtttgttc 1380

aaaaaactcc ttgtttctac t 1401

<210> SEQ ID NO 9
<211> LENGTH: 1690
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 9

ttaaccactc aagatggaag caataccact aataactata ctactagtag taacagcaag 60

caatgcagac aaaatctgca tcggctacca atcaacaaac tccacagaaa ccgtagacac 120

gctaacagaa aacaatgttc ctgtgacaca tgccaaagaa ttgctccaca cagagcacaa 180

tgggatgctg tgtgcaacaa atctgggacg tcctcttatt ctagacactt gcaccattga 240

aggactgatc tatggcaacc cttcttgtga tctactgttg ggaggaagag aatggtccta 300

catcgtcgaa agaccatcgg ctgttaatgg aatgtgttac cccgggaatg tagaaaacct 360

agaggaacta aggtcatttt ttagttctgc tagttcctac caaagaatcc agatctttcc 420

agacacaatc tggaatgtgt cttacagtgg aacaagcaaa gcatgttcag attcattcta 480

caggagcatg agatggttga ctcaaaagaa caacgcttac cctattcaag acgcccaata 540

cacaaataat agaggaaaga gcattctttt catgtggggc ataaatcacc cacctaccga 600

-continued

tactgcacag acaaatctgt acacaaggac tgacacaaca acaagtgtgg caacagaaga 660

tataaatagg accttcaaac cagtgatagg gccaaggccc cttgtcaatg gtctgcaggg 720

aagaattgat tattattggt cggtattgaa accaggtcag acattgcgag taagatccaa 780

tgggaatcta atcgctccat ggtatgggca cattctttca ggagagagcc acggaagaat 840

cctgaagact gatttaaaca gtggtagctg tgtagtgcaa tgtcaaacag aaagaggtgg 900

cttaaatact actttgccat tccacaatgt cagtaaatat gcatttggaa actgcccaaa 960

atatgttgga gtaaagagtc tcaaactggc agttggtctg aggaatgtgc ctgctagatc 1020

aagtagagga ctatttgggg ccatagctgg attcatagag ggaggttggt cagggctggt 1080

cgctggttgg tatgggttcc agcattcaaa tgatcaaggg gttggtatag ctgcagatag 1140

agactcaact caaagggcaa ttgacaaaat aacgtccaaa gtgaataata tagtcgataa 1200

aatgaacaag caatatgaaa ttattgatca tgaattcagc gaggttgaaa atagactcaa 1260

tatgatcaat aataagattg atgaccagat acaagacata tgggcatata acgctgaatt 1320

gctagtgctg cttgaaaacc agaaaacact cgatgagcat gatgcgaatg taaacaatct 1380

atataacaaa gtgaagaggg cactgggttc caatgcaatg gaagatggga aaggatgttt 1440

cgagctatac cataaatgtg atgatcagtg catggagaca attcggaacg ggacctataa 1500

caggaggaag tataaagagg aatcaagact agaaagacag aaaatagaag gggtcaagct 1560

ggaatctgaa ggaacttaca aaatcctcac catttattcg actgtcgcct catctcttgt 1620

gattgcaatg gggtttgctg ccttcttgtt ctgggccatg tccaatggat cttgcagatg 1680

caacatttga 1690

<210> SEQ ID NO 10
<211> LENGTH: 1428
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 10

aaatgaatcc aaatcagaag ataatagcaa ttggctctgt ttctctaact attgcgacaa 60

tatgcctcct catgcagatt gctatcttag caacgactat gacactacat ttcaagcaga 120

atgaatgcat caactcctcg aataatcaag tagtgccatg tgaaccaatc ataatagaaa 180

ggaacataac agagatagtg catttgaata gtactacctt agagaaggaa atttgtccta 240

aagtagcaga ctacaggaat tggtcaaaac cacaatgtca aatcacaggg ttcgctcctt 300

tctccaagga caattcaatt aggctctccg caggtggaga tatttgggtg acaagagaac 360

cttatgtatc gtgcggtctt ggtaaatgtt atcaatttgc acttgggcag ggaaccactt 420

tggagaacaa acactcaaac ggcacagcac atgatagaac tcctcataga acccttttaa 480

tgaatgagtt gggtgttccg tttcatttgg caaccaaaca agtgtgcata gcatggtcca 540

gctcaagctg ccatgatggg aaagcatggt tacatgtttg tgtcactggg gatgatagaa 600

atgcaacggc tagcatcatt tatgatggga tacttgttga cagtattggt tcatggtcta 660

aaaacatcct cagaactcag gagtcagaat gcgtttgcat caatggaacc tgtgcagtag 720

taatgactga tggaagtgca tcaggaaggg ctgacactag aatactattt attagagagg 780

ggaaaattgc acacattagc ccattgtcag gaagtgctca gcatgtggag gaatgctcct 840

gttacccccg atatccagaa gttagatgtg tttgcagaga caattggaag ggatccaata 900

ggcccgttct atatataaat atggcaaatt atagtattga ttccagttat gtgtgctcag 960

gacttgttgg cgacacacca agaaatgatg ataggtctag cagcagcaac tgcagagatc 1020

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ctaataacga gagaggggcc ccaggagtaa aagggtgggc ctttgacaat ggaaatgaca 1080

tttggatggg aagaacaatc aaaaaggatt cgcgctcagg ttatgagact ttcagggtca 1140

ttggtggttg gaccactgct aattccaagt cacagataaa tagacaagtc atagttgaca 1200

gtgataactc gtctgggtat tctggtatct tctctgttga aggcaaaagc tgcatcaaca 1260

ggtgtttttа cgtggagttg ataagaggaa gaccaaagga gactagggtg tggtggactt 1320

caaatagcat cattgtattt tgtggaactt caggtaccta tggaacaggc tcatggcctg 1380

atggggcgaa tatcaatttc atgcctatat aagctttcgc aattttag 1428

<210> SEQ ID NO 11
<211> LENGTH: 564
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 11

Met Glu Lys Ile Val Leu Leu Phe Ala Ile Val Ser Leu Val Lys Ser
1 5 10 15

Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
20 25 30

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
35 40 45

Leu Glu Lys Lys His Asn Gly Lys Leu Cys Asp Leu Asp Gly Val Lys
50 55 60

Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
65 70 75 80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val
85 90 95

Glu Lys Ala Asn Pro Val Asn Asp Leu Cys Tyr Pro Gly Asp Phe Asn
100 105 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
115 120 125

Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Ser His Glu Ala Ser
130 135 140

Leu Gly Val Ser Ser Ala Cys Pro Tyr Gln Gly Lys Ser Ser Phe Phe
145 150 155 160

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Ser Thr Tyr Pro Thr Ile
165 170 175

Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp
180 185 190

Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Lys Leu Tyr Gln
195 200 205

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg
210 215 220

Leu Val Pro Arg Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Ser Gly
225 230 235 240

Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn
245 250 255

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
260 265 270

Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Leu Glu Tyr Gly
275 280 285

Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
290 295 300

Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys

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305                 310                 315                 320

Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser
                325                 330                 335

Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile
            340                 345                 350

Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His
        355                 360                 365

Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln
    370                 375                 380

Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asp Lys
385                 390                 395                 400

Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu
                405                 410                 415

Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp
            420                 425                 430

Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg
        435                 440                 445

Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val
    450                 455                 460

Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe
465                 470                 475                 480

Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Val Arg Asn
                485                 490                 495

Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg
            500                 505                 510

Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly Ile Tyr Gln Ile
        515                 520                 525

Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile Met
    530                 535                 540

Val Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser Leu Gln Cys
545                 550                 555                 560

Arg Ile Cys Ile


<210> SEQ ID NO 12
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 12

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Met Val
1               5                   10                  15

Thr Gly Ile Val Ser Leu Met Leu Gln Ile Gly Asn Met Ile Ser Ile
            20                  25                  30

Trp Val Ser His Ser Ile His Thr Gly Asn Gln His Gln Ser Glu Pro
        35                  40                  45

Ile Ser Asn Thr Asn Phe Leu Thr Glu Lys Ala Val Ala Ser Val Lys
    50                  55                  60

Leu Ala Gly Asn Ser Ser Leu Cys Pro Ile Asn Gly Trp Ala Val Tyr
65                  70                  75                  80

Ser Lys Asp Asn Ser Ile Arg Ile Gly Ser Lys Gly Asp Val Phe Val
                85                  90                  95

Ile Arg Glu Pro Phe Ile Ser Cys Ser His Leu Glu Cys Arg Thr Phe
            100                 105                 110

Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His Ser Asn Gly Thr
        115                 120                 125
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Val Lys Asp Arg Ser Pro His Arg Thr Leu Met Ser Cys Pro Val Gly
    130                 135                 140

Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser Val Ala Trp Ser
145                 150                 155                 160

Ala Ser Ala Cys His Asp Gly Thr Ser Trp Leu Thr Ile Gly Ile Ser
                165                 170                 175

Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr Asn Gly Ile Ile
            180                 185                 190

Thr Asp Thr Ile Lys Ser Trp Arg Asn Asn Ile Leu Arg Thr Gln Glu
        195                 200                 205

Ser Glu Cys Ala Cys Val Asn Gly Ser Cys Phe Thr Val Met Thr Asp
    210                 215                 220

Gly Pro Ser Asn Gly Gln Ala Ser His Lys Ile Phe Lys Met Glu Lys
225                 230                 235                 240

Gly Lys Val Val Lys Ser Val Glu Leu Asp Ala Pro Asn Tyr His Tyr
                245                 250                 255

Glu Glu Cys Ser Cys Tyr Pro Asn Ala Gly Glu Ile Thr Cys Val Cys
            260                 265                 270

Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val Ser Phe Asn Gln
        275                 280                 285

Asn Leu Glu Tyr Gln Ile Gly Tyr Ile Cys Ser Gly Val Phe Gly Asp
    290                 295                 300

Asn Pro Arg Pro Asn Asp Gly Thr Gly Ser Cys Gly Pro Val Ser Ser
305                 310                 315                 320

Asn Gly Ala Tyr Gly Val Lys Gly Phe Ser Phe Lys Tyr Gly Asn Gly
                325                 330                 335

Val Trp Ile Gly Arg Thr Lys Ser Thr Asn Ser Arg Ser Gly Phe Glu
            340                 345                 350

Met Ile Trp Asp Pro Asn Gly Trp Thr Glu Thr Asp Ser Ser Phe Ser
        355                 360                 365

Val Lys Gln Asp Ile Val Ala Ile Thr Asp Trp Ser Gly Tyr Ser Gly
    370                 375                 380

Ser Phe Val Gln His Pro Glu Leu Thr Gly Leu Asp Cys Ile Arg Pro
385                 390                 395                 400

Cys Phe Trp Val Glu Leu Ile Arg Gly Arg Pro Lys Glu Ser Thr Ile
                405                 410                 415

Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val Asn Ser Asp Thr
            420                 425                 430

Val Gly Trp Ser Trp Pro Asp Gly Ala Glu Leu Pro Phe Thr Ile Asp
        435                 440                 445

Lys


<210> SEQ ID NO 13
<211> LENGTH: 564
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 13

Met Glu Lys Ile Val Leu Leu Phe Ala Ile Val Ser Leu Val Lys Ser
1               5                   10                  15

Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
            20                  25                  30

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
        35                  40                  45
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Leu Glu Lys Thr His Asn Gly Lys Leu Cys Asp Leu Asp Gly Val Lys
    50                  55                  60

Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
65                  70                  75                  80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val
                85                  90                  95

Glu Lys Ala Asn Pro Ala Asn Asp Leu Cys Tyr Pro Gly Asp Phe Asn
            100                 105                 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
        115                 120                 125

Lys Ile Gln Ile Ile Pro Lys Asn Ser Trp Ser Ser His Glu Ala Ser
    130                 135                 140

Leu Gly Val Ser Ser Ala Cys Pro Tyr Gln Gly Lys Ser Ser Phe Phe
145                 150                 155                 160

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Asn Ala Tyr Pro Thr Ile
                165                 170                 175

Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp
            180                 185                 190

Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Arg Leu Tyr Gln
        195                 200                 205

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg
    210                 215                 220

Leu Val Pro Lys Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Asn Gly
225                 230                 235                 240

Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn
                245                 250                 255

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
            260                 265                 270

Val Lys Lys Gly Asp Ser Ala Ile Met Lys Ser Glu Leu Glu Tyr Gly
        275                 280                 285

Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
    290                 295                 300

Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys
305                 310                 315                 320

Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser
                325                 330                 335

Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile
            340                 345                 350

Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His
        355                 360                 365

Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln
    370                 375                 380

Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asp Lys
385                 390                 395                 400

Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu
                405                 410                 415

Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp
            420                 425                 430

Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg
        435                 440                 445

Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val
    450                 455                 460

Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe
465                 470                 475                 480
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Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Val Arg Asn
                485                 490                 495

Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg
            500                 505                 510

Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Ile Gly Thr Tyr Gln Ile
        515                 520                 525

Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile Met
    530                 535                 540

Val Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser Leu Gln Cys
545                 550                 555                 560

Arg Ile Cys Ile


<210> SEQ ID NO 14
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 14

Met Asn Pro Asn Gln Lys Ile Thr Thr Ile Gly Ser Ile Cys Met Val
1               5                   10                  15

Ile Gly Ile Val Ser Leu Met Leu Gln Ile Gly Asn Ile Ile Ser Ile
            20                  25                  30

Trp Val Ser His Ser Ile Gln Thr Gly Asn Gln His Gln Ala Glu Pro
        35                  40                  45

Cys Asn Gln Ser Ile Ile Thr Tyr Glu Asn Asn Thr Trp Val Asn Gln
    50                  55                  60

Thr Tyr Val Asn Ile Ser Asn Thr Asn Phe Leu Thr Glu Lys Ala Val
65                  70                  75                  80

Ala Ser Val Thr Leu Ala Gly Asn Ser Ser Leu Cys Pro Ile Ser Gly
                85                  90                  95

Trp Ala Val Tyr Ser Lys Asp Asn Gly Ile Arg Ile Gly Ser Lys Gly
            100                 105                 110

Asp Val Phe Val Ile Arg Glu Pro Phe Ile Ser Cys Ser His Leu Glu
        115                 120                 125

Cys Arg Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His
    130                 135                 140

Ser Asn Gly Thr Val Lys Asp Arg Ser Pro His Arg Thr Leu Met Ser
145                 150                 155                 160

Cys Pro Val Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser
                165                 170                 175

Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Thr Ser Trp Leu Thr
            180                 185                 190

Ile Gly Ile Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr
        195                 200                 205

Asn Gly Ile Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Asn Ile Met
    210                 215                 220

Arg Thr Gln Glu Ser Glu Cys Ala Cys Val Asn Gly Ser Cys Phe Thr
225                 230                 235                 240

Val Met Thr Asp Gly Pro Ser Asn Gly Gln Ala Ser Tyr Lys Ile Phe
                245                 250                 255

Arg Ile Glu Lys Gly Lys Val Val Lys Ser Ala Glu Leu Asn Ala Pro
            260                 265                 270

Asn Tyr His Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Ala Gly Glu Ile
        275                 280                 285
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Thr Cys Val Cys Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val
    290                 295                 300

Ser Phe Asn Gln Asn Leu Glu Tyr Arg Ile Gly Tyr Ile Cys Ser Gly
305                 310                 315                 320

Val Phe Gly Asp Asn Pro Arg Pro Asn Asp Gly Thr Gly Ser Cys Gly
                325                 330                 335

Pro Val Ser Pro Lys Gly Ala Tyr Gly Ile Lys Gly Phe Ser Phe Lys
            340                 345                 350

Tyr Gly Asn Gly Val Trp Ile Gly Arg Thr Lys Ser Thr Asn Ser Arg
        355                 360                 365

Ser Gly Phe Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Gly Thr Asp
    370                 375                 380

Ser Asn Phe Ser Val Lys Gln Asp Ile Val Ala Ile Thr Asp Trp Ser
385                 390                 395                 400

Gly Tyr Ser Gly Ser Phe Val Gln His Pro Glu Leu Thr Gly Leu Asp
                405                 410                 415

Cys Ile Arg Pro Cys Phe Trp Val Glu Leu Ile Arg Gly Arg Pro Lys
            420                 425                 430

Glu Ser Thr Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val
        435                 440                 445

Asn Ser Asp Thr Val Gly Trp Ser Trp Pro Asp Gly Ala Glu Leu Pro
    450                 455                 460

Phe Thr Ile Asp Lys
465
```

<210> SEQ ID NO 15
<211> LENGTH: 564
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 15

```
Met Glu Lys Ile Val Leu Leu Leu Ala Thr Val Ser Leu Val Lys Ser
1               5                   10                  15

Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
            20                  25                  30

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
        35                  40                  45

Leu Glu Arg Thr His Asn Gly Lys Leu Cys Asp Leu Asn Gly Val Lys
    50                  55                  60

Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
65                  70                  75                  80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val
                85                  90                  95

Glu Lys Ala Ser Pro Ala Asn Asp Leu Cys Tyr Pro Gly Asn Phe Asn
            100                 105                 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
        115                 120                 125

Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Asn His Asp Ala Ser
    130                 135                 140

Ser Gly Val Ser Ser Ala Cys Pro Tyr Leu Gly Arg Ser Ser Phe Phe
145                 150                 155                 160

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Ser Ser Tyr Pro Thr Ile
                165                 170                 175

Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp
            180                 185                 190
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```
Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Arg Leu Tyr Gln
        195                 200                 205

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg
    210                 215                 220

Leu Val Pro Glu Ile Ala Thr Arg Pro Lys Val Asn Gly Gln Ser Gly
225                 230                 235                 240

Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn
                245                 250                 255

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
            260                 265                 270

Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Leu Glu Tyr Gly
        275                 280                 285

Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
    290                 295                 300

Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys
305                 310                 315                 320

Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Thr
                325                 330                 335

Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile
            340                 345                 350

Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His
        355                 360                 365

Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Glu Ser Thr Gln
    370                 375                 380

Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asn Lys
385                 390                 395                 400

Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu
                405                 410                 415

Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp
            420                 425                 430

Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg
        435                 440                 445

Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val
    450                 455                 460

Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe
465                 470                 475                 480

Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Val Lys Asn
                485                 490                 495

Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Asn Arg
            500                 505                 510

Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Met Gly Thr Tyr Gln Ile
        515                 520                 525

Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile Met
    530                 535                 540

Val Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser Leu Gln Cys
545                 550                 555                 560

Arg Ile Cys Ile


<210> SEQ ID NO 16
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 16

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Met Val
```

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```
1               5                   10                  15

Val Gly Ile Ile Ser Leu Met Leu Gln Ile Gly Asn Thr Ile Ser Val
            20                  25                  30

Trp Val Ser His Ile Ile Lys Thr Trp His Pro Asn Gln Pro Glu Pro
        35                  40                  45

Cys Asn Gln Ser Ile Asn Phe Tyr Thr Glu Gln Ala Ala Ala Ser Val
    50                  55                  60

Thr Leu Ala Gly Asn Ser Ser Leu Cys Pro Ile Ser Gly Trp Ala Ile
65                  70                  75                  80

Tyr Ser Lys Asp Asn Ser Ile Arg Ile Gly Ser Lys Gly Asp Val Phe
                85                  90                  95

Val Ile Arg Glu Pro Phe Ile Ser Cys Ser His Leu Glu Cys Arg Thr
            100                 105                 110

Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His Ser Asn Gly
        115                 120                 125

Thr Val Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser Cys Pro Val
    130                 135                 140

Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser Val Ala Trp
145                 150                 155                 160

Ser Ala Ser Ala Cys His Asp Gly Ile Ser Trp Leu Thr Ile Gly Ile
                165                 170                 175

Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr Asn Gly Ile
            180                 185                 190

Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Asn Thr Leu Arg Thr Gln
        195                 200                 205

Glu Ser Glu Cys Ala Cys Val Asn Gly Ser Cys Phe Thr Val Met Thr
    210                 215                 220

Asp Gly Pro Ser Asn Glu Gln Ala Ser Tyr Lys Ile Phe Lys Ile Glu
225                 230                 235                 240

Lys Gly Arg Val Val Lys Ser Val Glu Leu Asn Ala Pro Asn Tyr His
                245                 250                 255

Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Ala Gly Glu Ile Thr Cys Val
            260                 265                 270

Cys Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val Ser Phe Asn
        275                 280                 285

Gln Asn Leu Glu Tyr Gln Ile Gly Tyr Ile Cys Ser Gly Val Phe Gly
    290                 295                 300

Asp Ser Pro Arg Pro Asn Asp Gly Thr Gly Ser Cys Gly Pro Val Ser
305                 310                 315                 320

Leu Asn Gly Ala Tyr Gly Val Lys Gly Phe Ser Phe Lys Tyr Gly Asn
                325                 330                 335

Gly Val Trp Ile Gly Arg Thr Lys Ser Thr Ser Ser Arg Ser Gly Phe
            340                 345                 350

Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Glu Thr Asp Ser Ser Phe
        355                 360                 365

Ser Leu Lys Gln Asp Ile Ile Ala Ile Thr Asp Trp Ser Gly Tyr Ser
    370                 375                 380

Gly Ser Phe Ile Gln His Pro Glu Leu Thr Gly Leu Asn Cys Met Arg
385                 390                 395                 400

Pro Cys Phe Trp Val Glu Leu Ile Arg Gly Arg Pro Lys Glu Lys Thr
                405                 410                 415

Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val Asn Ser Asp
            420                 425                 430
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```
Thr Val Gly Trp Ser Trp Pro Asp Gly Ala Glu Leu Pro Tyr Thr Ile
        435                 440                 445

Asp Lys
    450


<210> SEQ ID NO 17
<211> LENGTH: 564
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 17

Met Glu Lys Ile Val Leu Leu Leu Ala Thr Val Ser Leu Val Lys Ser
1               5                   10                  15

Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
            20                  25                  30

Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
        35                  40                  45

Leu Glu Arg Thr His Asn Gly Lys Leu Cys Asp Leu Asn Gly Val Lys
    50                  55                  60

Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
65                  70                  75                  80

Pro Met Cys Asp Glu Phe Ile Asn Val Pro Glu Trp Ser Tyr Ile Val
                85                  90                  95

Glu Lys Ala Ser Pro Ala Asn Asp Leu Cys Tyr Pro Gly Asn Phe Asn
            100                 105                 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu
        115                 120                 125

Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Asn His Asp Ala Ser
    130                 135                 140

Ser Gly Val Ser Ser Ala Cys Pro Tyr Leu Gly Arg Ser Ser Phe Phe
145                 150                 155                 160

Arg Asn Val Val Trp Leu Ile Lys Lys Asn Ser Ser Tyr Pro Thr Ile
                165                 170                 175

Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp
            180                 185                 190

Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Arg Leu Tyr Gln
        195                 200                 205

Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg
    210                 215                 220

Leu Val Ser Glu Ile Ala Thr Arg Pro Lys Val Asn Gly Gln Ser Gly
225                 230                 235                 240

Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn
                245                 250                 255

Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Tyr Ala Tyr Lys Ile
            260                 265                 270

Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Leu Glu Tyr Gly
        275                 280                 285

Asn Cys Asn Thr Lys Cys Gln Thr Pro Met Gly Ala Ile Asn Ser Ser
    290                 295                 300

Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys
305                 310                 315                 320

Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Thr
                325                 330                 335

Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile
            340                 345                 350
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```
Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His
        355                 360                 365

Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Glu Ser Thr Gln
    370                 375                 380

Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asn Lys
385                 390                 395                 400

Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu
                405                 410                 415

Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp
            420                 425                 430

Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg
        435                 440                 445

Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val
    450                 455                 460

Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe
465                 470                 475                 480

Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Val Lys Asn
                485                 490                 495

Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Asn Arg
            500                 505                 510

Glu Glu Ile Ser Gly Val Lys Leu Glu Ser Met Gly Thr Tyr Gln Ile
        515                 520                 525

Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile Met
    530                 535                 540

Val Ala Gly Leu Ser Leu Trp Met Cys Ser Asn Gly Ser Leu Gln Cys
545                 550                 555                 560

Arg Ile Cys Ile


<210> SEQ ID NO 18
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 18

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Met Val
1               5                   10                  15

Val Gly Ile Ile Ser Leu Met Leu Gln Ile Gly Asn Thr Ile Ser Val
            20                  25                  30

Trp Val Ser His Ile Ile Lys Thr Trp His Pro Asn Gln Pro Glu Pro
        35                  40                  45

Cys Asn Gln Ser Ile Asn Phe Tyr Thr Glu Gln Ala Ala Ala Ser Val
    50                  55                  60

Thr Leu Ala Gly Asn Ser Ser Leu Cys Pro Ile Ser Gly Trp Ala Ile
65                  70                  75                  80

Tyr Ser Lys Asp Asn Ser Ile Arg Ile Gly Ser Lys Gly Asp Val Phe
                85                  90                  95

Val Ile Arg Glu Pro Phe Ile Ser Cys Ser His Leu Glu Cys Arg Thr
            100                 105                 110

Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His Ser Asn Gly
        115                 120                 125

Thr Val Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser Cys Pro Val
    130                 135                 140

Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser Val Ala Trp
145                 150                 155                 160

Ser Ala Ser Ala Cys His Asp Gly Ile Ser Trp Leu Thr Ile Gly Ile
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                165                 170                 175

Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr Asn Gly Ile
            180                 185                 190

Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Asn Thr Leu Arg Thr Gln
        195                 200                 205

Glu Ser Glu Cys Ala Cys Val Asn Gly Ser Cys Phe Thr Val Met Thr
    210                 215                 220

Asp Gly Pro Ser Asn Glu Gln Ala Ser Tyr Lys Ile Phe Lys Ile Glu
225                 230                 235                 240

Lys Gly Arg Val Val Lys Ser Val Glu Leu Asn Ala Pro Asn Tyr His
                245                 250                 255

Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Ala Gly Glu Ile Thr Cys Val
            260                 265                 270

Cys Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val Ser Phe Asn
        275                 280                 285

Gln Asn Leu Glu Tyr Gln Ile Gly Tyr Ile Cys Ser Gly Val Phe Gly
    290                 295                 300

Asp Ser Pro Arg Pro Asn Asp Gly Thr Gly Ser Cys Gly Pro Val Ser
305                 310                 315                 320

Leu Asn Gly Ala Tyr Gly Val Lys Gly Phe Ser Phe Lys Tyr Gly Asn
                325                 330                 335

Gly Val Trp Ile Gly Arg Thr Lys Ser Thr Ser Ser Arg Ser Gly Phe
            340                 345                 350

Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Glu Thr Asp Ser Ser Phe
        355                 360                 365

Ser Leu Lys Gln Asp Ile Ile Ala Ile Thr Asp Trp Ser Gly Tyr Ser
    370                 375                 380

Gly Ser Phe Ile Gln His Pro Glu Leu Thr Gly Leu Asn Cys Met Arg
385                 390                 395                 400

Pro Cys Phe Trp Val Glu Leu Ile Arg Gly Arg Pro Lys Glu Lys Thr
                405                 410                 415

Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val Asn Ser Asp
            420                 425                 430

Thr Val Gly Trp Ser Trp Pro Asp Gly Ala Glu Leu Pro Tyr Thr Ile
        435                 440                 445

Asp Lys
    450


<210> SEQ ID NO 19
<211> LENGTH: 558
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 19

Met Glu Ala Ile Pro Leu Ile Thr Ile Leu Leu Val Val Thr Ala Ser
1               5                   10                  15

Asn Ala Asp Lys Ile Cys Ile Gly Tyr Gln Ser Thr Asn Ser Thr Glu
            20                  25                  30

Thr Val Asp Thr Leu Thr Glu Asn Asn Val Pro Val Thr His Ala Lys
        35                  40                  45

Glu Leu Leu His Thr Glu His Asn Gly Met Leu Cys Ala Thr Asn Leu
    50                  55                  60

Gly Arg Pro Leu Ile Leu Asp Thr Cys Thr Ile Glu Gly Leu Ile Tyr
65                  70                  75                  80

Gly Asn Pro Ser Cys Asp Leu Leu Leu Gly Gly Arg Glu Trp Ser Tyr
```

-continued

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                85                  90                  95

Ile Val Glu Arg Pro Ser Ala Val Asn Gly Met Cys Tyr Pro Gly Asn
            100                 105                 110

Val Glu Asn Leu Glu Glu Leu Arg Ser Phe Phe Ser Ser Ala Ser Ser
        115                 120                 125

Tyr Gln Arg Ile Gln Ile Phe Pro Asp Thr Ile Trp Asn Val Ser Tyr
    130                 135                 140

Ser Gly Thr Ser Lys Ala Cys Ser Asp Ser Phe Tyr Arg Ser Met Arg
145                 150                 155                 160

Trp Leu Thr Gln Lys Asn Asn Ala Tyr Pro Ile Gln Asp Ala Gln Tyr
                165                 170                 175

Thr Asn Asn Arg Gly Lys Ser Ile Leu Phe Met Trp Gly Ile Asn His
            180                 185                 190

Pro Pro Thr Asp Thr Ala Gln Thr Asn Leu Tyr Thr Arg Thr Asp Thr
        195                 200                 205

Thr Thr Ser Val Ala Thr Glu Asp Ile Asn Arg Thr Phe Lys Pro Val
    210                 215                 220

Ile Gly Pro Arg Pro Leu Val Asn Gly Leu Gln Gly Arg Ile Asp Tyr
225                 230                 235                 240

Tyr Trp Ser Val Leu Lys Pro Gly Gln Thr Leu Arg Val Arg Ser Asn
                245                 250                 255

Gly Asn Leu Ile Ala Pro Trp Tyr Gly His Ile Leu Ser Gly Glu Ser
            260                 265                 270

His Gly Arg Ile Leu Lys Thr Asp Leu Asn Ser Gly Ser Cys Val Val
        275                 280                 285

Gln Cys Gln Thr Glu Arg Gly Gly Leu Asn Thr Thr Leu Pro Phe His
    290                 295                 300

Asn Val Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Val Gly Val
305                 310                 315                 320

Lys Ser Leu Lys Leu Ala Val Gly Leu Arg Asn Val Pro Ala Arg Ser
                325                 330                 335

Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp
            340                 345                 350

Ser Gly Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln
        355                 360                 365

Gly Val Gly Ile Ala Ala Asp Arg Asp Ser Thr Gln Arg Ala Ile Asp
    370                 375                 380

Lys Ile Thr Ser Lys Val Asn Asn Ile Val Asp Lys Met Asn Lys Gln
385                 390                 395                 400

Tyr Glu Ile Ile Asp His Glu Phe Ser Glu Val Glu Asn Arg Leu Asn
                405                 410                 415

Met Ile Asn Asn Lys Ile Asp Asp Gln Ile Gln Asp Ile Trp Ala Tyr
            420                 425                 430

Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu
        435                 440                 445

His Asp Ala Asn Val Asn Asn Leu Tyr Asn Lys Val Lys Arg Ala Leu
    450                 455                 460

Gly Ser Asn Ala Met Glu Asp Gly Lys Gly Cys Phe Glu Leu Tyr His
465                 470                 475                 480

Lys Cys Asp Asp Gln Cys Met Glu Thr Ile Arg Asn Gly Thr Tyr Asn
                485                 490                 495

Arg Arg Lys Tyr Lys Glu Glu Ser Arg Leu Glu Arg Gln Lys Ile Glu
            500                 505                 510
```

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```
Gly Val Lys Leu Glu Ser Glu Gly Thr Tyr Lys Ile Leu Thr Ile Tyr
        515                 520                 525

Ser Thr Val Ala Ser Ser Leu Val Ile Ala Met Gly Phe Ala Ala Phe
    530                 535                 540

Leu Phe Trp Ala Met Ser Asn Gly Ser Cys Arg Cys Asn Ile
545                 550                 555


<210> SEQ ID NO 20
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 20

Met Asn Pro Asn Gln Lys Ile Ile Ala Ile Gly Ser Val Ser Leu Thr
1               5                   10                  15

Ile Ala Thr Ile Cys Leu Leu Met Gln Ile Ala Ile Leu Ala Thr Thr
            20                  25                  30

Met Thr Leu His Phe Lys Gln Asn Glu Cys Ile Asn Ser Ser Asn Asn
        35                  40                  45

Gln Val Val Pro Cys Glu Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu
    50                  55                  60

Ile Val His Leu Asn Ser Thr Thr Leu Glu Lys Glu Ile Cys Pro Lys
65                  70                  75                  80

Val Ala Asp Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
                85                  90                  95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
            100                 105                 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Gly Leu Gly Lys
        115                 120                 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Glu Asn Lys His
    130                 135                 140

Ser Asn Gly Thr Ala His Asp Arg Thr Pro His Arg Thr Leu Leu Met
145                 150                 155                 160

Asn Glu Leu Gly Val Pro Phe His Leu Ala Thr Lys Gln Val Cys Ile
                165                 170                 175

Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
            180                 185                 190

Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Ile Ile Tyr Asp
        195                 200                 205

Gly Ile Leu Val Asp Ser Ile Gly Ser Trp Ser Lys Asn Ile Leu Arg
    210                 215                 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Ala Val Val
225                 230                 235                 240

Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe
                245                 250                 255

Ile Arg Glu Gly Lys Ile Ala His Ile Ser Pro Leu Ser Gly Ser Ala
            260                 265                 270

Gln His Val Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Glu Val Arg
        275                 280                 285

Cys Val Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Leu Tyr
    290                 295                 300

Ile Asn Met Ala Asn Tyr Ser Ile Asp Ser Ser Tyr Val Cys Ser Gly
305                 310                 315                 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Arg Ser Ser Ser Ser Asn
                325                 330                 335
```

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```
Cys Arg Asp Pro Asn Asn Glu Arg Gly Ala Pro Gly Val Lys Gly Trp
            340                 345                 350

Ala Phe Asp Asn Gly Asn Asp Ile Trp Met Gly Arg Thr Ile Lys Lys
        355                 360                 365

Asp Ser Arg Ser Gly Tyr Glu Thr Phe Arg Val Ile Gly Gly Trp Thr
    370                 375                 380

Thr Ala Asn Ser Lys Ser Gln Ile Asn Arg Gln Val Ile Val Asp Ser
385                 390                 395                 400

Asp Asn Ser Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser
                405                 410                 415

Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Lys
            420                 425                 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Ile Val Phe Cys Gly
        435                 440                 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
    450                 455                 460

Asn Phe Met Pro Ile
465
```

<210> SEQ ID NO 21
<211> LENGTH: 1737
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 21

```
agcaaaagca ggggatacaa aatgaacact caaatcctgg tattcgctct ggtggcgagc     60

attccgacaa atgcagacaa gatctgcctt gggcatcatg ccgtgtcaaa cgggactaaa    120

gtaaacacat taactgagag aggagtggaa gtcgttaatg caactgaaac ggtggaacga    180

acaaacgttc ccaggatctg ctcaaaaggg aaaaggacag ttgacctcgg tcaatgtgga    240

cttctgggaa caatcactgg gccaccccaa tgtgaccaat tcctagaatt ttcggccgac    300

ttaattattg agaggcgaga aggaagtgat gtctgttatc ctgggaaatt cgtgaatgaa    360

gaagctctga ggcaaattct cagagagtca ggcggaattg acaaggagac aatgggattc    420

acctacagcg gaataagaac taatggaaca accagtgcat gtaggagatc aggatcttca    480

ttctatgcag agatgaaatg gctcctgtca aacacagaca atgctgcttt cccgcaaatg    540

actaagtcat acaagaacac aaggaaagac ccagctctga taatatgggg gatccaccat    600

tccggatcaa ctacagaaca gaccaagcta tatgggagtg gaaacaaact gataacagtt    660

gggagttcta attaccaaca gtcctttgta ccgagtccag gagcgagacc acaagtgaat    720

ggccaatctg gaagaattga ctttcattgg ctgatactaa accctaatga cacggtcact    780

ttcagtttca atggggcctt catagctcca gaccgtgcaa gctttctgag agggaagtcc    840

atgggaattc agagtgaagt acaggttgat gccaattgtg aaggagattg ctatcatagt    900

ggagggacaa taataagtaa tttgcccttt cagaacataa atagcagggc agtaggaaaa    960

tgtccgagat atgttaagca agagagtctg ctgttggcaa caggaatgaa gaatgttccc   1020

gaaatcccaa agaggaggag gagaggccta tttggtgcta tagcgggttt cattgaaaat   1080

ggatgggaag gtttgattga tgggtggtat ggcttcaggc atcaaaatgc acaaggggag   1140

ggaactgctg cagattacaa aagcacccaa tcagcaattg atcaaataac agggaaatta   1200

aatcggctta tagaaaaaac taaccaacag tttgagttaa tagacaacga attcactgag   1260

gttgaaaggc aaattggcaa tgtgataaac tggaccagag attccatgac agaagtgtgg   1320

tcctataacg ctgaactctt agtagcaatg gagaatcagc acacaattga tctggccgac   1380
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tcagaaatga acaaactgta cgaacgagtg aagagacaac tgagagagaa tgccgaagaa 1440

gatggcactg gttgcttcga aatatttcac aagtgtgatg acgactgcat ggccagtatt 1500

agaaacaaca cctatgatca cagcaagtac agggaagaag caatacaaaa tagaatacag 1560

attgacccag tcaaactaag cagcggctac aaagatgtga tactttggtt tagcttcggg 1620

gcatcatgtt tcatacttct ggccattgca atgggccttg tcttcatatg tgtgaagaat 1680

ggaaacatgc ggtgcactat ttgtatataa gtttggaaaa acacccttgt ttctact 1737

<210> SEQ ID NO 22
<211> LENGTH: 1465
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 22

agcaaaagca gggtgatcga gaatgaatcc aaatcagaaa ctatttgcat tatctggagt 60

ggcaatagca cttagtgtac tgaacttatt gataggaatc tcaaacgtcg gattgaacgt 120

atctctacat ctaaaggaaa aaggacccaa acaggaggag aatttaacat gcacgaccat 180

taatcaaaac aacactactg tagtagaaaa cacatatgta aataatacaa caataattac 240

caagggaact gatttgaaaa caccaagcta tctgctgttg aacaagagcc tgtgcaatgt 300

tgaagggtgg gtcgtgatag caaaagacaa tgcagtaaga tttggggaaa gtgaacaaat 360

cattgttacc agggagccat atgtatcatg cgacccaaca ggatgcaaaa tgtatgcctt 420

gcaccaaggg actaccatta ggaacaaaca ttcaaatgga acgattcatg acagaacagc 480

tttcagaggt ctcatctcca ctccattggg cactccacca accgtaagta acagtgactt 540

tatgtgtgtt ggatggtcaa gcacaacttg ccatgatggg attgctagga tgactatctg 600

tatacaagga aataatgaca atgctacagc aacggtttat tacaacagaa ggctgaccac 660

taccattaag acctgggcca gaaacattct gaggactcaa gaatcagaat gtgtgtgcca 720

caatggcaca tgtgcagttg taatgaccga cggatcggct agtagtcaag cctatacaaa 780

agtaatgtat ttccacaagg gattagtagt taaggaggag gagttaaggg gatcagccag 840

acatattgag gaatgctcct gttatggaca caatcaaaag gtgacctgtg tgtgcagaga 900

taactggcag ggagcaaaca ggcctattat agaaattgat atgagcacat tggagcacac 960

aagtagatac gtgtgcactg gaattctcac agacaccagc agacctgggg acaaatctag 1020

tggtgattgt tccaatccaa taactgggag tcccggcgtt ccgggagtga agggattcgg 1080

gtttctaaat ggggataaca catggcttgg taggaccatc agccccagat caagaagtgg 1140

attcgaaatg ttgaaaatac ctaatgcagg tactgatccc aattctagaa tagcagaacg 1200

acaggaaatt gtcgacaata acaattggtc aggctattcc ggaagcttta ttgactattg 1260

gaatgataac agtgaatgct acaatccatg cttttacgta gagttaatta gaggaagacc 1320

cgaagaggct aaatacgtat ggtgggcaag taacagtcta attgccctat gtggaagccc 1380

attcccagtt gggtctggtt ccttccccga tggggcacaa atccaatact tttcgtaaaa 1440

tgcaaaaaaa ctccttgttt ctact 1465

<210> SEQ ID NO 23
<211> LENGTH: 1754
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 23

agcaaaagca ggggatacaa aatgaatact caaattttgg cattcattgc ttgtatgctg 60

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attggaacta aaggagacaa aatatgtctt gggcaccatg ctgtggcaaa tgggacaaaa 120

gtgaacacac taacagagag gggaattgaa gtagtcaatg ccacggagac ggtggaaact 180

gtaaatatta aaaaaatatg cactcaagga aaaaggccaa cagatctggg acaatgtgga 240

cttctaggaa ccctaatagg acctccccaa tgcgatcaat ttctggagtt tgacgctaat 300

ttgataattg aacgaagaga aggaaccgat gtgtgctatc ccgggaagtt cacaaatgaa 360

gaatcactga ggcagatcct tcgagggtca ggaggaattg ataaagagtc aatgggtttc 420

acctatagtg gaataagaac caatggggcg acgagtgcct gcagaagatc aggttcttct 480

ttctatgcgg agatgaagtg gttactgtcg aattcagaca atgcggcatt tccccaaatg 540

actaagtcgt ataggaatcc caggaacaaa ccagctctga taatctgggg agtgcatcac 600

tctggatcag ctactgagca gaccaaactc tatggaagtg gaaacaagtt gataacagta 660

ggaagctcga aataccagca atcattcact ccaagtccgg gagcacggcc acaagtgaat 720

ggacaatcag gaaggattga ttttcattgg ctactccttg accccaatga cacagtgacc 780

ttcactttca atggggcatt catagcccct gacagggcaa gtttctttag aggagaatcg 840

ctaggagtcc agagtgatgt tcctttggat tctggttgtg aaggggattg cttccacagt 900

gggggtacga tagtcagttc cctgccattc caaaacatca accctagaac agtggggaaa 960

tgccctcgat atgtcaaaca gacaagcctc cttttggcta caggaatgag aaacgtccca 1020

gagaacccca agcaggccta ccggaaacgg atgaccagag gccttttgg agcgattgct 1080

ggattcatag agaatggatg ggaaggtctc atcgatggat ggtatggttt cagacatcaa 1140

aatgcacaag gagaaggaac tgcagctgac tacaaaagca cccaatctgc aatagatcag 1200

atcacaggca aattgaatcg tctgattgac aaaacaaacc agcagtttga actgatagac 1260

aatgaattca gtgagataga acaacaaatc gggaatgtca ttaactggac acgagactca 1320

atgactgagg tatggtcgta taatgctgag ctgttggtgg caatggagaa tcagcataca 1380

atagatcttg cagactcaga aatgaacaaa ctttacgaac gcgtcagaaa acaactaagg 1440

gaaaatgctg aagaagatgg aactggatgc tttgagatat tccataagtg tgatgatcag 1500

tgtatggaga gcataaggaa caacacttat gaccataccc aatacaggac agagtcattg 1560

cagaatagaa tacagataga cccagtgaaa ttgagtagtg gatacaaaga cataatctta 1620

tggtttagct tcggggcatc atgttttctt cttctagcca ttgcaatggg attggttttc 1680

atttgcataa agaatggaaa catgcggtgc actatttgta tatagtttga gaaaaaaaca 1740

cccttgtttc tact 1754

<210> SEQ ID NO 24
<211> LENGTH: 1453
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 24

agcaaaagca ggtgcgagat gaatccgaat cagaagataa taacaatcgg ggtagtgaat 60

accactctgt caacaatagc ccttctcatt ggagtgggaa acttagtttt caacacagtc 120

atacatgaga aaataggaga ccatcaaata gtgacccatc caacaataat gacccctgaa 180

gtaccgaact gcagtgacac tataataaca tacaataaca ctgttataaa caacataaca 240

acaacaataa taactgaagc agaaaggcct ttcaagtctc cactaccgct gtgccccttc 300

agaggattct tccctttca caaggacaat gcaatacgac tgggtgaaaa caaagacgtc 360

atagtcacaa gggagcctta tgttagctgc gataatgaca actgctggtc ctttgctctc 420

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gcacaaggag cattgctagg gactaaacat agcaatggga ccattaaaga cagaacacca 480

tataggtctc taattcgttt cccaatagga acagctccag tactaggaaa ttacaaagag 540

atatgcattg cttggtcgag cagcagttgc tttgacggga aagagtggat gcatgtgtgc 600

atgacaggga atgataatga tgcaagtgcc cagataatat atggaggaag aatgacagac 660

tccattaaat catggaggaa ggacatacta agaacccagg agtctgaatg tcaatgcatt 720

gacgggactt gtgttgttgc tgtcacagat ggccctgctg ctaatagtgc agatcacagg 780

gtttactgga tacgggaggg aagaataata aagtatgaaa atgttcccaa aacaaagata 840

caacacttag aagaatgttc ctgctatgtg gacattgatg tttactgtat atgtagggac 900

aattggaagg gctctaacag accttggatg agaatcaaca acgagactat actggaaaca 960

ggatatgtat gtagtaaatt tcactcagac acccccaggc cagctgaccc ttcaataatg 1020

tcatgtgact ccccaagcaa tgtcaatgga ggacccggag tgaaggggtt tggtttcaaa 1080

gctggcaatg atgtatggtt aggtagaaca gtgtcaacta gtggtagatc gggctttgaa 1140

attatcaaag ttacagaagg gtggatcaac tctcctaacc atgtcaaatc aattacacaa 1200

acactagtgt ccaacaatga ctggtcaggc tattcaggta gcttcattgt caaagccaag 1260

gactgttttc agccctgttt ttatgttgag cttatacgag ggaggcccaa caagaatgat 1320

gacgtctctt ggacaagtaa tagtatagtt actttctgtg gactagacaa tgaacctgga 1380

tcgggaaatt ggccagatgg ttctaacatt gggtttatgc ccaagtaata gaaaaaagca 1440

ccttgtttct act 1453

<210> SEQ ID NO 25
<211> LENGTH: 1733
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 25

agcgaaagca ggggatacaa aatgaatact caaattttgg cattcattgc ttgtatgctg 60

attggaacta aaggagacaa aatatgtctt gggcaccatg ctgtggcaaa tgggacaaaa 120

gtgaacacac taacagagag gggaattgaa gtagtcaatg ccacggagac ggtggaaact 180

gtaaatatta agaaaatatg cactcaagga aaaaggccaa cagatctggg acaatgtgga 240

cttctaggaa ccctaatagg acctccccaa tgcgatcaat ttctggagtt tgacgctaat 300

ttgataattg aacgaagaga aggaaccgat gtgtgctatc ccgggaagtt cacaaatgaa 360

gaatcactga ggcagatcct tcgagggtca ggaggaattg ataaagagtc aatgggtttc 420

acctatagtg gaataagaac caatggggcg acgagtgcct gcagaagatc aggttcttct 480

ttctatgcgg agatgaagtg gttactgtcg aattcagaca atgcggcatt tccccaaatg 540

actaagtcgt ataggaatcc caggaacaaa ccagctctga taatctgggg agtgcatcac 600

tctggatcag ctactgagca gaccaaactc tatggaagtg gaaacaagtt gataacagta 660

ggaagctcga aataccagca atcattcact ccaagtccgg gagcacggcc acaagtgaat 720

ggacaatcag gaaggattga ttttcattgg ctactccttg accccaatga cacagtgacc 780

ttcactttca atggggcatt catagcccct gacagggcaa gtttctttag aggagaatcg 840

ctaggagtcc agagtgatgt tcctttggat tctggttgtg aaggggattg cttccacagt 900

gggggtacga tagtcagttc cctgccattc caaaacatca accctagaac agtggggaaa 960

tgccctcgat atgtcaaaca gacaagcctc cttttggcta caggaatgag aaacgtccca 1020

gagaacccca agaccagagg ccttttgga gcgattgctg gattcataga gaatggatgg 1080

-continued

gaaggtctca tcgatggatg gtatggtttc agacatcaaa atgcacaagg agaaggaact 1140

gcagctgact acaaaagcac ccaatctgca atagatcaga tcacaggcaa attgaatcgt 1200

ctgattgaca aaacaaacca gcagtttgaa ctgatagaca atgaattcag tgagatagaa 1260

caacaaatcg ggaatgtcat taactggaca cgagactcaa tgactgaggt atggtcgtat 1320

aatgctgagc tgttggtggc aatggagaat cagcatacaa tagatcttgc agactcagaa 1380

atgaacaaac tttacgaacg cgtcagaaaa caactaaggg aaaatgctga agaagatgga 1440

actggatgct ttgagatatt ccataagtgt gatgatcagt gtatggagag cataaggaac 1500

aacacttatg accataccca atacaggaca gagtcattgc agaatagaat acagatagac 1560

ccagtgaaat tgagtagtgg atacaaagac ataatcttat ggtttagctt cggggcatca 1620

tgtttttcttc ttctagccat tgcaatggga ttggttttca tttgcataaa gaatggaaac 1680

atgcggtgca ctatttgtat atagtttgag aaaaaaacac ccttgtttct act 1733

<210> SEQ ID NO 26
<211> LENGTH: 1453
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 26

agcaaaagca ggtgcgagat gaatccgaat cagaagataa taacaatcgg ggtagtgaat 60

accactctgt caacaatagc ccttctcatt ggagtgggaa acttagtttt caacacagtc 120

atacatgaga aaataggaga ccatcaaata gtgacccatc caacaataat gacccctgaa 180

gtaccgaact gcagtgacac tataataaca tacaataaca ctgttataaa caacataaca 240

acaacaataa taactgaagc agaaaggcct ttcaagtctc cactaccgct gtgccccttc 300

agaggattct tccctttca caaggacaat gcaatacgac tgggtgaaaa caaagacgtc 360

atagtcacaa gggagcctta tgttagctgc gataatgaca actgctggtc ctttgctctc 420

gcacaaggag cattgctagg gactaaacat agcaatggga ccattaaaga cagaacacca 480

tataggtctc taattcgttt cccaatagga acagctccag tactaggaaa ttacaaagag 540

atatgcattg cttggtcgag cagcagttgc tttgacggga aagagtggat gcatgtgtgc 600

atgacaggga atgataatga tgcaagtgcc cagataatat atggaggaag aatgacagac 660

tccattaaat catggaggaa ggacatacta agaacccagg agtctgaatg tcaatgcatt 720

gacgggactt gtgttgttgc tgtcacagat ggccctgctg ctaatagtgc agatcacagg 780

gtttactgga tacgggaggg aagaataata aagtatgaaa atgttcccaa aacaaagata 840

caacacttag aagaatgttc ctgctatgtg gacattgatg tttactgtat atgtagggac 900

aattggaagg gctctaacag accttggatg agaatcaaca acgagactat actggaaaca 960

ggatatgtat gtagtaaatt tcactcagac acccccaggc cagctgaccc ttcaataatg 1020

tcatgtgact ccccaagcaa tgtcaatgga ggacccggag tgaaggggtt tggtttcaaa 1080

gctggcaatg atgtatggtt aggtagaaca gtgtcaacta gtggtagatc gggctttgaa 1140

attatcaaag ttacagaagg gtggatcaac tctcctaacc atgtcaaatc aattacacaa 1200

acactagtgt ccaacaatga ctggtcaggc tattcaggta gcttcattgt caaagccaag 1260

gactgttttc agccctgttt ttatgttgag cttatacgag ggaggcccaa caagaatgat 1320

gacgtctctt ggacaagtaa tagtatagtt actttctgtg gactagacaa tgaacctgga 1380

tcgggaaatt ggccagatgg ttctaacatt gggtttatgc ccaagtaata gaaaaaagca 1440

ccttgtttct act 1453

<210> SEQ ID NO 27
<211> LENGTH: 562
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 27

Met Asn Thr Gln Ile Leu Val Phe Ala Leu Val Ala Ser Ile Pro Thr
1 5 10 15

Asn Ala Asp Lys Ile Cys Leu Gly His His Ala Val Ser Asn Gly Thr
20 25 30

Lys Val Asn Thr Leu Thr Glu Arg Gly Val Glu Val Val Asn Ala Thr
35 40 45

Glu Thr Val Glu Arg Thr Asn Val Pro Arg Ile Cys Ser Lys Gly Lys
50 55 60

Arg Thr Val Asp Leu Gly Gln Cys Gly Leu Leu Gly Thr Ile Thr Gly
65 70 75 80

Pro Pro Gln Cys Asp Gln Phe Leu Glu Phe Ser Ala Asp Leu Ile Ile
85 90 95

Glu Arg Arg Glu Gly Ser Asp Val Cys Tyr Pro Gly Lys Phe Val Asn
100 105 110

Glu Glu Ala Leu Arg Gln Ile Leu Arg Glu Ser Gly Gly Ile Asp Lys
115 120 125

Glu Thr Met Gly Phe Thr Tyr Ser Gly Ile Arg Thr Asn Gly Thr Thr
130 135 140

Ser Ala Cys Arg Arg Ser Gly Ser Ser Phe Tyr Ala Glu Met Lys Trp
145 150 155 160

Leu Leu Ser Asn Thr Asp Asn Ala Ala Phe Pro Gln Met Thr Lys Ser
165 170 175

Tyr Lys Asn Thr Arg Lys Asp Pro Ala Leu Ile Ile Trp Gly Ile His
180 185 190

His Ser Gly Ser Thr Thr Glu Gln Thr Lys Leu Tyr Gly Ser Gly Asn
195 200 205

Lys Leu Ile Thr Val Gly Ser Ser Asn Tyr Gln Gln Ser Phe Val Pro
210 215 220

Ser Pro Gly Ala Arg Pro Gln Val Asn Gly Gln Ser Gly Arg Ile Asp
225 230 235 240

Phe His Trp Leu Ile Leu Asn Pro Asn Asp Thr Val Thr Phe Ser Phe
245 250 255

Asn Gly Ala Phe Ile Ala Pro Asp Arg Ala Ser Phe Leu Arg Gly Lys
260 265 270

Ser Met Gly Ile Gln Ser Glu Val Gln Val Asp Ala Asn Cys Glu Gly
275 280 285

Asp Cys Tyr His Ser Gly Gly Thr Ile Ile Ser Asn Leu Pro Phe Gln
290 295 300

Asn Ile Asn Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val Lys Gln
305 310 315 320

Glu Ser Leu Leu Leu Ala Thr Gly Met Lys Asn Val Pro Glu Ile Pro
325 330 335

Lys Arg Arg Arg Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu
340 345 350

Asn Gly Trp Glu Gly Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln
355 360 365

Asn Ala Gln Gly Glu Gly Thr Ala Ala Asp Tyr Lys Ser Thr Gln Ser
370 375 380

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Ala Ile Asp Gln Ile Thr Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr
385                 390                 395                 400

Asn Gln Gln Phe Glu Leu Ile Asp Asn Glu Phe Thr Glu Val Glu Arg
                405                 410                 415

Gln Ile Gly Asn Val Ile Asn Trp Thr Arg Asp Ser Met Thr Glu Val
            420                 425                 430

Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Met Glu Asn Gln His Thr
        435                 440                 445

Ile Asp Leu Ala Asp Ser Glu Met Asn Lys Leu Tyr Glu Arg Val Lys
    450                 455                 460

Arg Gln Leu Arg Glu Asn Ala Glu Glu Asp Gly Thr Gly Cys Phe Glu
465                 470                 475                 480

Ile Phe His Lys Cys Asp Asp Asp Cys Met Ala Ser Ile Arg Asn Asn
                485                 490                 495

Thr Tyr Asp His Ser Lys Tyr Arg Glu Glu Ala Ile Gln Asn Arg Ile
            500                 505                 510

Gln Ile Asp Pro Val Lys Leu Ser Ser Gly Tyr Lys Asp Val Ile Leu
        515                 520                 525

Trp Phe Ser Phe Gly Ala Ser Cys Phe Ile Leu Leu Ala Ile Ala Met
    530                 535                 540

Gly Leu Val Phe Ile Cys Val Lys Asn Gly Asn Met Arg Cys Thr Ile
545                 550                 555                 560

Cys Ile


<210> SEQ ID NO 28
<211> LENGTH: 471
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 28

Met Asn Pro Asn Gln Lys Leu Phe Ala Leu Ser Gly Val Ala Ile Ala
1               5                   10                  15

Leu Ser Val Leu Asn Leu Leu Ile Gly Ile Ser Asn Val Gly Leu Asn
            20                  25                  30

Val Ser Leu His Leu Lys Glu Lys Gly Pro Lys Gln Glu Glu Asn Leu
        35                  40                  45

Thr Cys Thr Thr Ile Asn Gln Asn Asn Thr Thr Val Val Glu Asn Thr
    50                  55                  60

Tyr Val Asn Asn Thr Thr Ile Ile Thr Lys Gly Thr Asp Leu Lys Thr
65                  70                  75                  80

Pro Ser Tyr Leu Leu Leu Asn Lys Ser Leu Cys Asn Val Glu Gly Trp
                85                  90                  95

Val Val Ile Ala Lys Asp Asn Ala Val Arg Phe Gly Glu Ser Glu Gln
            100                 105                 110

Ile Ile Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Thr Gly Cys
        115                 120                 125

Lys Met Tyr Ala Leu His Gln Gly Thr Thr Ile Arg Asn Lys His Ser
    130                 135                 140

Asn Gly Thr Ile His Asp Arg Thr Ala Phe Arg Gly Leu Ile Ser Thr
145                 150                 155                 160

Pro Leu Gly Thr Pro Pro Thr Val Ser Asn Ser Asp Phe Met Cys Val
                165                 170                 175

Gly Trp Ser Ser Thr Thr Cys His Asp Gly Ile Ala Arg Met Thr Ile
            180                 185                 190
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Cys Ile Gln Gly Asn Asn Asp Asn Ala Thr Ala Thr Val Tyr Tyr Asn
        195                 200                 205

Arg Arg Leu Thr Thr Thr Ile Lys Thr Trp Ala Arg Asn Ile Leu Arg
    210                 215                 220

Thr Gln Glu Ser Glu Cys Val Cys His Asn Gly Thr Cys Ala Val Val
225                 230                 235                 240

Met Thr Asp Gly Ser Ala Ser Ser Gln Ala Tyr Thr Lys Val Met Tyr
                245                 250                 255

Phe His Lys Gly Leu Val Val Lys Glu Glu Glu Leu Arg Gly Ser Ala
            260                 265                 270

Arg His Ile Glu Glu Cys Ser Cys Tyr Gly His Asn Gln Lys Val Thr
        275                 280                 285

Cys Val Cys Arg Asp Asn Trp Gln Gly Ala Asn Arg Pro Ile Ile Glu
    290                 295                 300

Ile Asp Met Ser Thr Leu Glu His Thr Ser Arg Tyr Val Cys Thr Gly
305                 310                 315                 320

Ile Leu Thr Asp Thr Ser Arg Pro Gly Asp Lys Ser Ser Gly Asp Cys
                325                 330                 335

Ser Asn Pro Ile Thr Gly Ser Pro Gly Val Pro Gly Val Lys Gly Phe
            340                 345                 350

Gly Phe Leu Asn Gly Asp Asn Thr Trp Leu Gly Arg Thr Ile Ser Pro
        355                 360                 365

Arg Ser Arg Ser Gly Phe Glu Met Leu Lys Ile Pro Asn Ala Gly Thr
    370                 375                 380

Asp Pro Asn Ser Arg Ile Ala Glu Arg Gln Glu Ile Val Asp Asn Asn
385                 390                 395                 400

Asn Trp Ser Gly Tyr Ser Gly Ser Phe Ile Asp Tyr Trp Asn Asp Asn
                405                 410                 415

Ser Glu Cys Tyr Asn Pro Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg
            420                 425                 430

Pro Glu Glu Ala Lys Tyr Val Trp Trp Ala Ser Asn Ser Leu Ile Ala
        435                 440                 445

Leu Cys Gly Ser Pro Phe Pro Val Gly Ser Gly Ser Phe Pro Asp Gly
    450                 455                 460

Ala Gln Ile Gln Tyr Phe Ser
465                 470


<210> SEQ ID NO 29
<211> LENGTH: 567
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 29

Met Asn Thr Gln Ile Leu Ala Phe Ile Ala Cys Met Leu Ile Gly Thr
1               5                   10                  15

Lys Gly Asp Lys Ile Cys Leu Gly His His Ala Val Ala Asn Gly Thr
            20                  25                  30

Lys Val Asn Thr Leu Thr Glu Arg Gly Ile Glu Val Val Asn Ala Thr
        35                  40                  45

Glu Thr Val Glu Thr Val Asn Ile Lys Lys Ile Cys Thr Gln Gly Lys
    50                  55                  60

Arg Pro Thr Asp Leu Gly Gln Cys Gly Leu Leu Gly Thr Leu Ile Gly
65                  70                  75                  80

Pro Pro Gln Cys Asp Gln Phe Leu Glu Phe Asp Ala Asn Leu Ile Ile
                85                  90                  95
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Glu Arg Arg Glu Gly Thr Asp Val Cys Tyr Pro Gly Lys Phe Thr Asn
            100                 105                 110

Glu Glu Ser Leu Arg Gln Ile Leu Arg Gly Ser Gly Gly Ile Asp Lys
        115                 120                 125

Glu Ser Met Gly Phe Thr Tyr Ser Gly Ile Arg Thr Asn Gly Ala Thr
    130                 135                 140

Ser Ala Cys Arg Arg Ser Gly Ser Ser Phe Tyr Ala Glu Met Lys Trp
145                 150                 155                 160

Leu Leu Ser Asn Ser Asp Asn Ala Ala Phe Pro Gln Met Thr Lys Ser
                165                 170                 175

Tyr Arg Asn Pro Arg Asn Lys Pro Ala Leu Ile Ile Trp Gly Val His
            180                 185                 190

His Ser Gly Ser Ala Thr Glu Gln Thr Lys Leu Tyr Gly Ser Gly Asn
        195                 200                 205

Lys Leu Ile Thr Val Gly Ser Ser Lys Tyr Gln Gln Ser Phe Thr Pro
    210                 215                 220

Ser Pro Gly Ala Arg Pro Gln Val Asn Gly Gln Ser Gly Arg Ile Asp
225                 230                 235                 240

Phe His Trp Leu Leu Leu Asp Pro Asn Asp Thr Val Thr Phe Thr Phe
                245                 250                 255

Asn Gly Ala Phe Ile Ala Pro Asp Arg Ala Ser Phe Phe Arg Gly Glu
            260                 265                 270

Ser Leu Gly Val Gln Ser Asp Val Pro Leu Asp Ser Gly Cys Glu Gly
        275                 280                 285

Asp Cys Phe His Ser Gly Gly Thr Ile Val Ser Ser Leu Pro Phe Gln
    290                 295                 300

Asn Ile Asn Pro Arg Thr Val Gly Lys Cys Pro Arg Tyr Val Lys Gln
305                 310                 315                 320

Thr Ser Leu Leu Leu Ala Thr Gly Met Arg Asn Val Pro Glu Asn Pro
                325                 330                 335

Lys Gln Ala Tyr Arg Lys Arg Met Thr Arg Gly Leu Phe Gly Ala Ile
            340                 345                 350

Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly Leu Ile Asp Gly Trp Tyr
        355                 360                 365

Gly Phe Arg His Gln Asn Ala Gln Gly Glu Gly Thr Ala Ala Asp Tyr
    370                 375                 380

Lys Ser Thr Gln Ser Ala Ile Asp Gln Ile Thr Gly Lys Leu Asn Arg
385                 390                 395                 400

Leu Ile Asp Lys Thr Asn Gln Gln Phe Glu Leu Ile Asp Asn Glu Phe
                405                 410                 415

Ser Glu Ile Glu Gln Gln Ile Gly Asn Val Ile Asn Trp Thr Arg Asp
            420                 425                 430

Ser Met Thr Glu Val Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Met
        435                 440                 445

Glu Asn Gln His Thr Ile Asp Leu Ala Asp Ser Glu Met Asn Lys Leu
    450                 455                 460

Tyr Glu Arg Val Arg Lys Gln Leu Arg Glu Asn Ala Glu Glu Asp Gly
465                 470                 475                 480

Thr Gly Cys Phe Glu Ile Phe His Lys Cys Asp Asp Gln Cys Met Glu
                485                 490                 495

Ser Ile Arg Asn Asn Thr Tyr Asp His Thr Gln Tyr Arg Thr Glu Ser
            500                 505                 510

Leu Gln Asn Arg Ile Gln Ile Asp Pro Val Lys Leu Ser Ser Gly Tyr
        515                 520                 525
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Lys Asp Ile Ile Leu Trp Phe Ser Phe Gly Ala Ser Cys Phe Leu Leu
    530                 535                 540

Leu Ala Ile Ala Met Gly Leu Val Phe Ile Cys Ile Lys Asn Gly Asn
545                 550                 555                 560

Met Arg Cys Thr Ile Cys Ile
                565


<210> SEQ ID NO 30
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 30

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Val Val Asn Thr Thr
1               5                   10                  15

Leu Ser Thr Ile Ala Leu Leu Ile Gly Val Gly Asn Leu Val Phe Asn
            20                  25                  30

Thr Val Ile His Glu Lys Ile Gly Asp His Gln Ile Val Thr His Pro
        35                  40                  45

Thr Ile Met Thr Pro Glu Val Pro Asn Cys Ser Asp Thr Ile Ile Thr
    50                  55                  60

Tyr Asn Asn Thr Val Ile Asn Asn Ile Thr Thr Thr Ile Ile Thr Glu
65                  70                  75                  80

Ala Glu Arg Pro Phe Lys Ser Pro Leu Pro Leu Cys Pro Phe Arg Gly
                85                  90                  95

Phe Phe Pro Phe His Lys Asp Asn Ala Ile Arg Leu Gly Glu Asn Lys
            100                 105                 110

Asp Val Ile Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Asn Asp Asn
        115                 120                 125

Cys Trp Ser Phe Ala Leu Ala Gln Gly Ala Leu Leu Gly Thr Lys His
    130                 135                 140

Ser Asn Gly Thr Ile Lys Asp Arg Thr Pro Tyr Arg Ser Leu Ile Arg
145                 150                 155                 160

Phe Pro Ile Gly Thr Ala Pro Val Leu Gly Asn Tyr Lys Glu Ile Cys
                165                 170                 175

Ile Ala Trp Ser Ser Ser Ser Cys Phe Asp Gly Lys Glu Trp Met His
            180                 185                 190

Val Cys Met Thr Gly Asn Asp Asn Asp Ala Ser Ala Gln Ile Ile Tyr
        195                 200                 205

Gly Gly Arg Met Thr Asp Ser Ile Lys Ser Trp Arg Lys Asp Ile Leu
    210                 215                 220

Arg Thr Gln Glu Ser Glu Cys Gln Cys Ile Asp Gly Thr Cys Val Val
225                 230                 235                 240

Ala Val Thr Asp Gly Pro Ala Ala Asn Ser Ala Asp His Arg Val Tyr
                245                 250                 255

Trp Ile Arg Glu Gly Arg Ile Ile Lys Tyr Glu Asn Val Pro Lys Thr
            260                 265                 270

Lys Ile Gln His Leu Glu Glu Cys Ser Cys Tyr Val Asp Ile Asp Val
        275                 280                 285

Tyr Cys Ile Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Trp Met
    290                 295                 300

Arg Ile Asn Asn Glu Thr Ile Leu Glu Thr Gly Tyr Val Cys Ser Lys
305                 310                 315                 320

Phe His Ser Asp Thr Pro Arg Pro Ala Asp Pro Ser Ile Met Ser Cys
                325                 330                 335
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Asp Ser Pro Ser Asn Val Asn Gly Gly Pro Gly Val Lys Gly Phe Gly
            340                 345                 350

Phe Lys Ala Gly Asn Asp Val Trp Leu Gly Arg Thr Val Ser Thr Ser
        355                 360                 365

Gly Arg Ser Gly Phe Glu Ile Ile Lys Val Thr Glu Gly Trp Ile Asn
    370                 375                 380

Ser Pro Asn His Val Lys Ser Ile Thr Gln Thr Leu Val Ser Asn Asn
385                 390                 395                 400

Asp Trp Ser Gly Tyr Ser Gly Ser Phe Ile Val Lys Ala Lys Asp Cys
                405                 410                 415

Phe Gln Pro Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Asn Lys
            420                 425                 430

Asn Asp Asp Val Ser Trp Thr Ser Asn Ser Ile Val Thr Phe Cys Gly
        435                 440                 445

Leu Asp Asn Glu Pro Gly Ser Gly Asn Trp Pro Asp Gly Ser Asn Ile
    450                 455                 460

Gly Phe Met Pro Lys
465


<210> SEQ ID NO 31
<211> LENGTH: 560
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 31

Met Asn Thr Gln Ile Leu Ala Phe Ile Ala Cys Met Leu Ile Gly Thr
1               5                   10                  15

Lys Gly Asp Lys Ile Cys Leu Gly His His Ala Val Ala Asn Gly Thr
            20                  25                  30

Lys Val Asn Thr Leu Thr Glu Arg Gly Ile Glu Val Val Asn Ala Thr
        35                  40                  45

Glu Thr Val Glu Thr Val Asn Ile Lys Lys Ile Cys Thr Gln Gly Lys
    50                  55                  60

Arg Pro Thr Asp Leu Gly Gln Cys Gly Leu Leu Gly Thr Leu Ile Gly
65                  70                  75                  80

Pro Pro Gln Cys Asp Gln Phe Leu Glu Phe Asp Ala Asn Leu Ile Ile
                85                  90                  95

Glu Arg Arg Glu Gly Thr Asp Val Cys Tyr Pro Gly Lys Phe Thr Asn
            100                 105                 110

Glu Glu Ser Leu Arg Gln Ile Leu Arg Gly Ser Gly Gly Ile Asp Lys
        115                 120                 125

Glu Ser Met Gly Phe Thr Tyr Ser Gly Ile Arg Thr Asn Gly Ala Thr
    130                 135                 140

Ser Ala Cys Arg Arg Ser Gly Ser Ser Phe Tyr Ala Glu Met Lys Trp
145                 150                 155                 160

Leu Leu Ser Asn Ser Asp Asn Ala Ala Phe Pro Gln Met Thr Lys Ser
                165                 170                 175

Tyr Arg Asn Pro Arg Asn Lys Pro Ala Leu Ile Ile Trp Gly Val His
            180                 185                 190

His Ser Gly Ser Ala Thr Glu Gln Thr Lys Leu Tyr Gly Ser Gly Asn
        195                 200                 205

Lys Leu Ile Thr Val Gly Ser Ser Lys Tyr Gln Gln Ser Phe Thr Pro
    210                 215                 220

Ser Pro Gly Ala Arg Pro Gln Val Asn Gly Gln Ser Gly Arg Ile Asp
225                 230                 235                 240
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Phe His Trp Leu Leu Leu Asp Pro Asn Asp Thr Val Thr Phe Thr Phe
                245                 250                 255

Asn Gly Ala Phe Ile Ala Pro Asp Arg Ala Ser Phe Phe Arg Gly Glu
            260                 265                 270

Ser Leu Gly Val Gln Ser Asp Val Pro Leu Asp Ser Gly Cys Glu Gly
        275                 280                 285

Asp Cys Phe His Ser Gly Gly Thr Ile Val Ser Ser Leu Pro Phe Gln
    290                 295                 300

Asn Ile Asn Pro Arg Thr Val Gly Lys Cys Pro Arg Tyr Val Lys Gln
305                 310                 315                 320

Thr Ser Leu Leu Leu Ala Thr Gly Met Arg Asn Val Pro Glu Asn Pro
                325                 330                 335

Lys Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly
            340                 345                 350

Trp Glu Gly Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala
        355                 360                 365

Gln Gly Glu Gly Thr Ala Ala Asp Tyr Lys Ser Thr Gln Ser Ala Ile
    370                 375                 380

Asp Gln Ile Thr Gly Lys Leu Asn Arg Leu Ile Asp Lys Thr Asn Gln
385                 390                 395                 400

Gln Phe Glu Leu Ile Asp Asn Glu Phe Ser Glu Ile Glu Gln Gln Ile
                405                 410                 415

Gly Asn Val Ile Asn Trp Thr Arg Asp Ser Met Thr Glu Val Trp Ser
            420                 425                 430

Tyr Asn Ala Glu Leu Leu Val Ala Met Glu Asn Gln His Thr Ile Asp
        435                 440                 445

Leu Ala Asp Ser Glu Met Asn Lys Leu Tyr Glu Arg Val Arg Lys Gln
    450                 455                 460

Leu Arg Glu Asn Ala Glu Glu Asp Gly Thr Gly Cys Phe Glu Ile Phe
465                 470                 475                 480

His Lys Cys Asp Asp Gln Cys Met Glu Ser Ile Arg Asn Asn Thr Tyr
                485                 490                 495

Asp His Thr Gln Tyr Arg Thr Glu Ser Leu Gln Asn Arg Ile Gln Ile
            500                 505                 510

Asp Pro Val Lys Leu Ser Ser Gly Tyr Lys Asp Ile Ile Leu Trp Phe
        515                 520                 525

Ser Phe Gly Ala Ser Cys Phe Leu Leu Leu Ala Ile Ala Met Gly Leu
    530                 535                 540

Val Phe Ile Cys Ile Lys Asn Gly Asn Met Arg Cys Thr Ile Cys Ile
545                 550                 555                 560


<210> SEQ ID NO 32
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 32

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Val Val Asn Thr Thr
1               5                   10                  15

Leu Ser Thr Ile Ala Leu Leu Ile Gly Val Gly Asn Leu Val Phe Asn
            20                  25                  30

Thr Val Ile His Glu Lys Ile Gly Asp His Gln Ile Val Thr His Pro
        35                  40                  45

Thr Ile Met Thr Pro Glu Val Pro Asn Cys Ser Asp Thr Ile Ile Thr
    50                  55                  60
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Tyr Asn Asn Thr Val Ile Asn Asn Ile Thr Thr Thr Ile Ile Thr Glu
65                  70                  75                  80

Ala Glu Arg Pro Phe Lys Ser Pro Leu Pro Leu Cys Pro Phe Arg Gly
                85                  90                  95

Phe Phe Pro Phe His Lys Asp Asn Ala Ile Arg Leu Gly Glu Asn Lys
            100                 105                 110

Asp Val Ile Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Asn Asp Asn
        115                 120                 125

Cys Trp Ser Phe Ala Leu Ala Gln Gly Ala Leu Leu Gly Thr Lys His
    130                 135                 140

Ser Asn Gly Thr Ile Lys Asp Arg Thr Pro Tyr Arg Ser Leu Ile Arg
145                 150                 155                 160

Phe Pro Ile Gly Thr Ala Pro Val Leu Gly Asn Tyr Lys Glu Ile Cys
                165                 170                 175

Ile Ala Trp Ser Ser Ser Ser Cys Phe Asp Gly Lys Glu Trp Met His
            180                 185                 190

Val Cys Met Thr Gly Asn Asp Asn Asp Ala Ser Ala Gln Ile Ile Tyr
        195                 200                 205

Gly Gly Arg Met Thr Asp Ser Ile Lys Ser Trp Arg Lys Asp Ile Leu
    210                 215                 220

Arg Thr Gln Glu Ser Glu Cys Gln Cys Ile Asp Gly Thr Cys Val Val
225                 230                 235                 240

Ala Val Thr Asp Gly Pro Ala Ala Asn Ser Ala Asp His Arg Val Tyr
                245                 250                 255

Trp Ile Arg Glu Gly Arg Ile Ile Lys Tyr Glu Asn Val Pro Lys Thr
            260                 265                 270

Lys Ile Gln His Leu Glu Glu Cys Ser Cys Tyr Val Asp Ile Asp Val
        275                 280                 285

Tyr Cys Ile Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Trp Met
    290                 295                 300

Arg Ile Asn Asn Glu Thr Ile Leu Glu Thr Gly Tyr Val Cys Ser Lys
305                 310                 315                 320

Phe His Ser Asp Thr Pro Arg Pro Ala Asp Pro Ser Ile Met Ser Cys
                325                 330                 335

Asp Ser Pro Ser Asn Val Asn Gly Gly Pro Gly Val Lys Gly Phe Gly
            340                 345                 350

Phe Lys Ala Gly Asn Asp Val Trp Leu Gly Arg Thr Val Ser Thr Ser
        355                 360                 365

Gly Arg Ser Gly Phe Glu Ile Ile Lys Val Thr Glu Gly Trp Ile Asn
    370                 375                 380

Ser Pro Asn His Val Lys Ser Ile Thr Gln Thr Leu Val Ser Asn Asn
385                 390                 395                 400

Asp Trp Ser Gly Tyr Ser Gly Ser Phe Ile Val Lys Ala Lys Asp Cys
                405                 410                 415

Phe Gln Pro Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Asn Lys
            420                 425                 430

Asn Asp Asp Val Ser Trp Thr Ser Asn Ser Ile Val Thr Phe Cys Gly
        435                 440                 445

Leu Asp Asn Glu Pro Gly Ser Gly Asn Trp Pro Asp Gly Ser Asn Ile
    450                 455                 460

Gly Phe Met Pro Lys
465
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<210> SEQ ID NO 33
<211> LENGTH: 1743
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 33

agcaaaagca ggggaaaatg attgcagtca ttataatagc ggtactggca acggccggaa 60

aatcagacaa gatctgcatt gggtatcatg ccaacaattc aacaacacaa gtggatacga 120

tacttgagaa gaatgtaacc gtcacacact cagttgaatt gctggagaac caaaaagaag 180

aaagattctg caagatcttg aacaaggccc ctctcgattt aagaggatgt accatagagg 240

gttggatctt ggggaatccc caatgcgacc tattgcttgg tgatcaaagc tggtcatata 300

tagtggaaag acctacagct caaaatggga tctgctaccc aggaattttg aatgaagtag 360

aagaactgaa ggcacttatt ggatcaggag aaagagtgga gagatttgaa atgtttccca 420

aaagtacatg ggcaggagta gacaccagca gtggggtaac aaaggcttgc ccttatacta 480

gtggttcgtc tttctacaga aacctcctat ggataataaa aaccaagtcc gcagcatatc 540

cagtaattaa gggaacctac aataacactg gaagccagcc aatcctctat ttctggggtg 600

tgcaccatcc tcctgacacc aatgagcaaa acactttgta tggctctggt gatcgatatg 660

tcaggatggg aactgaaagc atgaattttg ccaagagccc agaaattgcg gcaaggcctg 720

ctgtgaatgg tcaaagaggc agaattgatt attactggtc tgttttaaag ccgggggaaa 780

ccttgaatgt ggaatctaat ggaaatctaa tcgccccttg gtatgcatac aaatttgtca 840

gcaccaatag taaaggagcc gtcttcaagt caaatttacc aatcgagaac tgtgatgcca 900

catgccagac tattgcagga gtcttaagaa ccaataaaac atttcagaat gtaagccctc 960

tgtggatagg agaatgcccc aaatatgtga aaagtgaaag tttgaggctt gcaactggac 1020

taagaaatat tccacagatt gagactagag gacttttcgg agctatcgca gggtttattg 1080

aaggaggatg gactggaatg atagatgggt ggtatggcta tcaccatgaa aattctcaag 1140

gctcagggta tgcggcagac agagaaagca ctcaaagggc tatagacgga attacaaata 1200

aggtcaattc cattatagac aaaatgaaca cacaattcga agctatagac cacgaattct 1260

caaatttgga gagaagaatt gacagtctga acaaaagaat ggaagatgga tttctggacg 1320

tttggacata caatgctgaa ctgttggttc ttcttgaaaa cgaaaggaca ctagacctac 1380

atgacgcgaa tgtgaagaac ctgtatgaaa aggtcaaatc acaactacgg gacaatgcta 1440

atgatctagg aaatggatgc tttgaatttt ggcataagtg tgacaatgaa tgcatagagt 1500

ctgtcaaaaa tggtacctat gactatccca aatatcagga tgaaagcaaa ttgaacaggc 1560

aggaaataga atcggtgaag ctggagaacc ttggtgtgta tcaaatcctc gccatttata 1620

gtacggtatc gagcagtcta gtcttggtag ggctgattat agcaatgggt ctttggatgt 1680

gttcaaatgg ttcaatgcaa tgcaggatat gtatataatt aagaaaaaca cccttgttct 1740

act 1743

<210> SEQ ID NO 34
<211> LENGTH: 1460
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 34

agcaaaagca gggtcaagat gaatccaaat cagaagattc tatgcacatc tgctactgcc 60

attgcaatag gcacaattgc tgtattaata ggaatagcaa acctgggttt gaacatagga 120

ctacacctga aaccgagctg caactgctcc aaccctcctc ctgaaacaac aaatgtaagc 180

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caaacaataa taaacaatta ctacaatgaa acaaatgtta cccaaataag taacacaaac 240

attcaacata tgggggaac cgaaaaggac ttcaacaatc tgactaaagg gctctgcaca 300

ataaattcat ggcatatatt cggaaaggac aatgctataa gaatagggga gaactctgat 360

gttttagtca caagagagcc atatgtttct tgtgatccag atgaatgcag attctatgct 420

ctcagccaag gaacaacaat acggggaaag cactcaaatg gaacaataca cgatagatcc 480

caataccgtg ctttagtgag ctggccttta tcatcaccac ccactgtgta caataccaga 540

gtagaatgca ttggatggtc cagtacaagc tgccatgatg ggaaagcacg aatgtctata 600

tgtgtctcag gtcccaacaa caatgcatca gcagtgattt ggtacaaagg gcggcctatc 660

acggaaatca atacgtgggc ccgaaacata ttgagaaccc aagaatctga gtgtgtatgc 720

cacaatggaa tatgtccagt agtgttcact gacggttctg ccaccggtcc agcagaaact 780

aggatatact atttcaaaga ggggaaaatc ctcaaatggg agccactaac tggaaccgcc 840

aagcacattg aagaatgctc ttgctatggg aaagactcag aaataacgtg cacatgtaga 900

gacaattggc aaggctcgaa tagaccagta atacaaataa accccacaat gatgactcac 960

actagtcaat acatatgcag ccctgtcctc acagacaatc cacgccccaa tgaccccacg 1020

gtaggcaagt gtaatgatcc ttatccagga aacaacaata atggagtcaa aggattctca 1080

tatttagatg gtgacaatac atggctagga agaacgataa gcacagcctc taggtctggg 1140

tatgaaatgc tgaaagtgcc taatgcattg acagatgata gatcaaaacc tactcaaggt 1200

cagacaattg tattaaacac agactggagt ggttacagtg ggtctttcat tgattactgg 1260

gcaaaagggg agtgctatag agcatgcttc tacgttgagc tgatccgtgg aaggccaaaa 1320

gaggacaaag tgtggtggac cagtaatagt atagtgtcga tgtgttccag cacagagttc 1380

cttggacaat ggaactggcc agatggggct aaaatagagt acttcctcta agatgtagaa 1440

aaaagaccct tgtttctact 1460

<210> SEQ ID NO 35
<211> LENGTH: 1747
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 35

agcaaaagca ggggaaaatg attgcaatca ttgtaatagc aatactggca gcagccggaa 60

aatcagacaa gatctgcatt gggtatcatg ccaacaattc aacaacacag gtagatacga 120

tacttgagaa gaatgtgact gtcacacact caattgaatt gctggaaaat cagaaggaag 180

aaagattctg caagatattg aacaaggccc ctctcgactt aagggaatgt accatagagg 240

gttggatctt ggggaatccc caatgcgacc tattgcttgg tgatcaaagc tggtcataca 300

ttgtggaaag acctactgct caaaacggga tctgctaccc aggaacctta aatgaggtag 360

aagaactgag ggcacttatt ggatcaggag aaagggtaga gagatttgag atgtttcccc 420

aaagcacctg gcaaggagtt gacaccaaca gtggaacaac aagatcctgc ccttattcta 480

ctggtgatcc gtctttctac agaaacctcc tatggataat aaaaaccaag acagcagaat 540

atccagtaat taagggaatt tacaacaaca ctggaaccca gccaatcctc tatttctggg 600

gtgtgcatca tcctcctaac accgacgagc aagatactct gtatggctct ggtgatcgat 660

acgttagaat gggaactgaa agcatgaatt ttgccaagag tccggaaatt gcggcaaggc 720

ctgctgtgaa tggacaaaga ggcagaattg attattattg gtcggtttta aaaccagggg 780

aaaccttgaa tgtggaatct aatggaaatc taatcgcccc ttggtatgca tacaaatttg 840

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tcaacacaaa tagtaaagga gccgtcttca ggtcagattt accaatcgag aactgcgatg 900

ccacatgcca gactattgca ggggttctaa ggaccaataa aacatttcag aatgtgagtc 960

ccctgtggat aggagaatgt cccaaatacg tgaaaagtga aagtctgagg cttgcaactg 1020

gactaagaaa tgttccacag attgaaacta gaggactctt cggagctatt gcagggttta 1080

ttgaaggagg atggactggg atgatagatg ggtggtatgg ctatcaccat gaaaattctc 1140

aagggtcagg atatgcagcg gacagagaaa gcactcaaaa ggctgtaaac agaattacaa 1200

ataaggtcaa ttccatcatc aacaaaatga acacacaatt tgaagctgtc gatcacgaat 1260

tttcaaatct ggagaggaga atcgacaatc tgaacaaaag aatgcaagat ggatttctgg 1320

atgtttggac atacaatgct gaactgttgg ttcttcttga aaacgaaaga acactagaca 1380

tgcatgacgc aaatgtgaag aacctacatg aaaaggtcaa atcacaacta agggacaatg 1440

ctaacgatct agggaatggt tgctttgaat tttggcataa gtgtgacaat gaatgcatag 1500

agtctgtcaa aaatggtaca tatgactatc ccaaatacca gactgaaagc aaattaaaca 1560

ggctaaaaat agaatcagta aagctagaga accttggtgt gtatcaaatt cttgccattt 1620

atagtacggt atcgagcagc ctagtgttgg tagggctgat catggcaatg ggtctttgga 1680

tgtgttcaaa tggttcaatg cagtgcaatg tgtgtatatg attaagaaaa acacccttgt 1740

ttctact 1747

<210> SEQ ID NO 36
<211> LENGTH: 1401
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 36

agcaaaagca ggagtttaac atgaatccaa atcagaagat aataaccatt gggtcaatct 60

gtatggtagt tggaataatc agcttgatgt tacaaattgg aaacataata tcaatatggg 120

ttagccacat aattcagact gggcatccaa accagcctgg gccatgcaat caaagcatca 180

atttttacac tgagcaggct gcagcttcag tgacattagc gggtaattcc tctctctgcc 240

ctattagtgg atgggctata tacagtaaag acaatagtat aagaattggt tccaaagggg 300

atgtgtttgt tatgagagaa ccattcgttt catgctccca tttggaatgc agaacctttt 360

tcttgactca aggagcccta ttgaatgaca agcattctaa tgggaccgtt aaagacagaa 420

gcccctatag aactttaatg agctgtcctg ttggtgaggc tccttcccca tacaactcaa 480

ggtttgagtc tgttgcttgg tcagcaagtg cttgccatga tggcattagt tggctaacaa 540

ttggaatttc cggtccggat aatggggctg tggctgtgtt gaaatacaat ggcataataa 600

cagacaccat caagagttgg aggaacaaca tactgaggac acaagagtct gaatgtgcat 660

gtgtgaatgg ttcttgtttt actgtaatga cagatggacc gagtaatgaa caggcctcat 720

acaagatttt caagatagag aaggggaaag tagtcaaatc agttgagttg aacgccccta 780

attatcatta cgaggaatgc tcctgttatc ctgatgctgg cgaaatcaca tgtgtgtgca 840

gggataattg gcatggctcg aaccgaccgt gggtgtcttt caatcagaat ctggagtatc 900

aaataggata tatatgcagt ggggttttcg gagacagtcc acgccccaat gatggaacag 960

gcagttgcgg tccagtgtct cttaacggag agtatggagt aaaagggttt tcatttaagt 1020

acggtgatgg tgtttggatc gggagaacca aaagcactag ttccaggagc gggtttgaaa 1080

tgatttggga tccaaatggg tggaccgaaa cagatagtaa cttctcattg aagcaagaca 1140

tcatagcaat aactgattgg tcaggataca gcgggagttt tgtccaacat ccagaactga 1200

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caggattaaa ttgcatgagg ccttgcttct gggttgaact aatcagaggg aggcccaaag 1260

agaaaacaat ctggactagt gggagcagta tatctttctg tggtgtaaat agtgacactg 1320

tgggttggtc ttggccagac ggtgctgagg tgccattcac cattgacaag tagtttgttc 1380

aaaaaactcc ttgtttctac t 1401

<210> SEQ ID NO 37
<211> LENGTH: 1745
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 37

agcaaaagca ggggaaaatg attgcaatca taatacttgc aatagtggtc tctaccagca 60

agtcagacag gatctgcatt ggttaccatg caaacaactc gacaacacaa gtggacacaa 120

tattagagaa gaatgtgaca gtgacacact cagtggagct cctagaaaac cagaaggaga 180

atagattctg cagagtcttg aataaagcgc cactggatct aatggactgc accactgagg 240

gttggatcct tggaaacccc cgatgtgata acttactcgg tgatcaaagt tggtcataca 300

tagtagagag gcctgatgcc caaaatggga tatgttaccc aggggtattg aaggagacgg 360

aagagctgaa agcactcatt gggtctatag atagcataca aagatttgaa atgtttccca 420

agagcacgtg gaccggggta gatactaata gcggagttac gagcgcttgc ccctacaatg 480

gtgaatcttc cttttacagg aatctgttgt ggataataaa aataagatct gatccgtact 540

cattgatcaa ggggacatat accaatacag gctctcagcc aatcttatat ttctggggtg 600

tgcaccatcc tccagatgaa gttgagcaag ctaacttgta tggaattggt acccggtatg 660

ttaggatggg aactgaaagt atgaattttg ccaaaggtcc tgaaatagca ggcagaccac 720

ctgcgaatgg gcaacgagga agaattgatt attattggtc tgtgttgaag ccaggagaaa 780

ccttgaatgt ggaatccaat ggaaatttaa tagctccttg gtatgcttac aagttcacta 840

gttccagaaa caagggagct attttcaaat cagaccttcc aattgagaat tgtgatgctg 900

tctgtcaaac tttagctgga gcaataaata caaacaaaac cttccaaaat attagtccag 960

tctggattgg agaatgcccc aaatatgtta aaagtaagag cctaaaacta gcaactggtc 1020

tgagaaatgt tccacaggca gaaacaagag gattgtttgg agcaatagct gggtttatag 1080

aaggaggatg gacaggtatg gtagacggat ggtacggata ccaccatgaa aattcacagg 1140

ggtctggtta tgcagcagat aaagaaagca ctcagaaagc aatagacggg atcaccaata 1200

aagtcaattc aatcattgac aaaatgaaca cacaatttga ggcagtagag catgagttct 1260

caagtctcga aaggagaata ggcaatctga acaaaagaat ggaagatgga tttttagacg 1320

tgtggacata caatgctgaa cttctggttc tactggaaaa tgagaggact ttggacatgc 1380

atgatgctaa tgtaaagaat ctacatgaaa aggtgaaatc acaattaagg gataatgcaa 1440

aggatttggg taatgggtgt tttgaatttt ggcacaaatg cgacaatgaa tgcatcaact 1500

cagttaaaaa tggcacatat gactacccaa agtaccagga agagagcaga cttaataggc 1560

aggaaataaa atcagtgatg ctggaaaatc tgggagtata ccaaatcctt gctatttata 1620

gtacggtatc gagcagtctg gttttggtgg gactgatcat tgccatgggt ctttggatgt 1680

gctcaaatgg ctcaatgcaa tgcaagatat gtatataatt agaaaaaaac acccttgttt 1740

ctact 1745

<210> SEQ ID NO 38
<211> LENGTH: 1467

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<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 38

agcaaaagca ggagtgaaaa tgaatccaaa tcagaggata ataacaattg gatccgtctc 60

tctaactatt gcaacagtgt gtttcctcat gcagattgcc atcctagcaa cgactgtgac 120

actgcatttc aaacaaaatg aatgcagcat tcccgcaaac aaccaagtaa cgccatgtga 180

accaatagta atagagagga acataacaga gatagtgtat ttgaataata ctaccataga 240

aaaagagatt tgtcctgaag tagtagaata caggaattgg tcaaaaccgc aatgtcaaat 300

tacagggttt gctcctttct ccaaggacaa ctcaattcgg ctttctgctg gtggggacat 360

ttggataaca agagaacctt atgtgtcatg cgaccccagt aaatgttatc aatttgcact 420

cgggcagggg accacgctgg acaacaaaca ctcaaatggc acaatacatg atagaatccc 480

tcatcggacc cttttgatga atgaattggg tgttccgttt catttgggaa ccaaacaagt 540

gtgcatagca tggtccagct caagctgtca tgatgggaaa gcatggttgc acgtttgtgt 600

cactggggat gatagaaatg caactgctag tttcatttat gatgggatgc ttattgacag 660

tattggttcc tggtctcaaa atatcctcag gactcaggag tcagaatgcg tttgtatcag 720

tggaacttgt acagtagtaa tgactgatgg aagtgcatca ggaagggcag acactagaat 780

actattcatt agagagggga aaattgtcca cattagtcca ttgtcaggaa gtgctcagca 840

tgtagaggaa tgttcttgtt atccccggta cccaaacgtc agatgtgtct gcagagacaa 900

ctggaagggc tctaataggc ccgttataga tataaatatg gcagattata gcattgactc 960

aagttatgtg tgctcaggac ttgttggaga cacaccaagg aacgatgata gctctagcag 1020

cagcaactgc agggatccta ataatgagag agggaaccca ggagtgaaag ggtgggcctt 1080

tgataatgga aatgatgtgt ggatgggaag aacaatcagt aaagattcgc gctcaggcta 1140

tgagaccttc aaggtcattg gtggttgggc cattgctaat tccaagtcac agaccaatag 1200

acaagtcata gttgataata acaactggtc tggttattct ggtattttct ctgttgaaag 1260

caaaggctgc atcaataggt gtttttatgt ggagttgata agaggaaggc cacaggagac 1320

tagagtatgg tggacctcaa acagtattgt cgtattttgt ggcacttcag ggacatatgg 1380

aacaggctca tggcctgatg gggcgaatat cgatttcatg cctatataag ctttcgcaat 1440

tttagaaaaa aactccttgt ttctact 1467

<210> SEQ ID NO 39
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 39

Met Ile Ala Val Ile Ile Ile Ala Val Leu Ala Thr Ala Gly Lys Ser
1 5 10 15

Asp Lys Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Thr Gln Val
20 25 30

Asp Thr Ile Leu Glu Lys Asn Val Thr Val Thr His Ser Val Glu Leu
35 40 45

Leu Glu Asn Gln Lys Glu Glu Arg Phe Cys Lys Ile Leu Asn Lys Ala
50 55 60

Pro Leu Asp Leu Arg Gly Cys Thr Ile Glu Gly Trp Ile Leu Gly Asn
65 70 75 80

Pro Gln Cys Asp Leu Leu Leu Gly Asp Gln Ser Trp Ser Tyr Ile Val
85 90 95

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Glu Arg Pro Thr Ala Gln Asn Gly Ile Cys Tyr Pro Gly Ile Leu Asn
            100                 105                 110

Glu Val Glu Glu Leu Lys Ala Leu Ile Gly Ser Gly Glu Arg Val Glu
        115                 120                 125

Arg Phe Glu Met Phe Pro Lys Ser Thr Trp Ala Gly Val Asp Thr Ser
    130                 135                 140

Ser Gly Val Thr Lys Ala Cys Pro Tyr Thr Ser Gly Ser Ser Phe Tyr
145                 150                 155                 160

Arg Asn Leu Leu Trp Ile Ile Lys Thr Lys Ser Ala Ala Tyr Pro Val
                165                 170                 175

Ile Lys Gly Thr Tyr Asn Asn Thr Gly Ser Gln Pro Ile Leu Tyr Phe
            180                 185                 190

Trp Gly Val His His Pro Pro Asp Thr Asn Glu Gln Asn Thr Leu Tyr
        195                 200                 205

Gly Ser Gly Asp Arg Tyr Val Arg Met Gly Thr Glu Ser Met Asn Phe
    210                 215                 220

Ala Lys Ser Pro Glu Ile Ala Ala Arg Pro Ala Val Asn Gly Gln Arg
225                 230                 235                 240

Gly Arg Ile Asp Tyr Tyr Trp Ser Val Leu Lys Pro Gly Glu Thr Leu
                245                 250                 255

Asn Val Glu Ser Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Tyr Lys
            260                 265                 270

Phe Val Ser Thr Asn Ser Lys Gly Ala Val Phe Lys Ser Asn Leu Pro
        275                 280                 285

Ile Glu Asn Cys Asp Ala Thr Cys Gln Thr Ile Ala Gly Val Leu Arg
    290                 295                 300

Thr Asn Lys Thr Phe Gln Asn Val Ser Pro Leu Trp Ile Gly Glu Cys
305                 310                 315                 320

Pro Lys Tyr Val Lys Ser Glu Ser Leu Arg Leu Ala Thr Gly Leu Arg
                325                 330                 335

Asn Ile Pro Gln Ile Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly
            340                 345                 350

Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly Tyr
        355                 360                 365

His His Glu Asn Ser Gln Gly Ser Gly Tyr Ala Ala Asp Arg Glu Ser
    370                 375                 380

Thr Gln Arg Ala Ile Asp Gly Ile Thr Asn Lys Val Asn Ser Ile Ile
385                 390                 395                 400

Asp Lys Met Asn Thr Gln Phe Glu Ala Ile Asp His Glu Phe Ser Asn
                405                 410                 415

Leu Glu Arg Arg Ile Asp Ser Leu Asn Lys Arg Met Glu Asp Gly Phe
            420                 425                 430

Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn
        435                 440                 445

Glu Arg Thr Leu Asp Leu His Asp Ala Asn Val Lys Asn Leu Tyr Glu
    450                 455                 460

Lys Val Lys Ser Gln Leu Arg Asp Asn Ala Asn Asp Leu Gly Asn Gly
465                 470                 475                 480

Cys Phe Glu Phe Trp His Lys Cys Asp Asn Glu Cys Ile Glu Ser Val
                485                 490                 495

Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Gln Asp Glu Ser Lys Leu
            500                 505                 510

Asn Arg Gln Glu Ile Glu Ser Val Lys Leu Glu Asn Leu Gly Val Tyr
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        515                 520                 525

Gln Ile Leu Ala Ile Tyr Ser Thr Val Ser Ser Ser Leu Val Leu Val
    530                 535                 540

Gly Leu Ile Ile Ala Met Gly Leu Trp Met Cys Ser Asn Gly Ser Met
545                 550                 555                 560

Gln Cys Arg Ile Cys Ile
                565


<210> SEQ ID NO 40
<211> LENGTH: 470
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 40

Met Asn Pro Asn Gln Lys Ile Leu Cys Thr Ser Ala Thr Ala Ile Ala
1               5                   10                  15

Ile Gly Thr Ile Ala Val Leu Ile Gly Ile Ala Asn Leu Gly Leu Asn
            20                  25                  30

Ile Gly Leu His Leu Lys Pro Ser Cys Asn Cys Ser Asn Pro Pro Pro
        35                  40                  45

Glu Thr Thr Asn Val Ser Gln Thr Ile Ile Asn Asn Tyr Tyr Asn Glu
    50                  55                  60

Thr Asn Val Thr Gln Ile Ser Asn Thr Asn Ile Gln His Met Gly Gly
65                  70                  75                  80

Thr Glu Lys Asp Phe Asn Asn Leu Thr Lys Gly Leu Cys Thr Ile Asn
                85                  90                  95

Ser Trp His Ile Phe Gly Lys Asp Asn Ala Ile Arg Ile Gly Glu Asn
            100                 105                 110

Ser Asp Val Leu Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Asp
        115                 120                 125

Glu Cys Arg Phe Tyr Ala Leu Ser Gln Gly Thr Thr Ile Arg Gly Lys
    130                 135                 140

His Ser Asn Gly Thr Ile His Asp Arg Ser Gln Tyr Arg Ala Leu Val
145                 150                 155                 160

Ser Trp Pro Leu Ser Ser Pro Pro Thr Val Tyr Asn Thr Arg Val Glu
                165                 170                 175

Cys Ile Gly Trp Ser Ser Thr Ser Cys His Asp Gly Lys Ala Arg Met
            180                 185                 190

Ser Ile Cys Val Ser Gly Pro Asn Asn Asn Ala Ser Ala Val Ile Trp
        195                 200                 205

Tyr Lys Gly Arg Pro Ile Thr Glu Ile Asn Thr Trp Ala Arg Asn Ile
    210                 215                 220

Leu Arg Thr Gln Glu Ser Glu Cys Val Cys His Asn Gly Ile Cys Pro
225                 230                 235                 240

Val Val Phe Thr Asp Gly Ser Ala Thr Gly Pro Ala Glu Thr Arg Ile
                245                 250                 255

Tyr Tyr Phe Lys Glu Gly Lys Ile Leu Lys Trp Glu Pro Leu Thr Gly
            260                 265                 270

Thr Ala Lys His Ile Glu Glu Cys Ser Cys Tyr Gly Lys Asp Ser Glu
        275                 280                 285

Ile Thr Cys Thr Cys Arg Asp Asn Trp Gln Gly Ser Asn Arg Pro Val
    290                 295                 300

Ile Gln Ile Asn Pro Thr Met Met Thr His Thr Ser Gln Tyr Ile Cys
305                 310                 315                 320

Ser Pro Val Leu Thr Asp Asn Pro Arg Pro Asn Asp Pro Thr Val Gly
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                325                 330                 335

Lys Cys Asn Asp Pro Tyr Pro Gly Asn Asn Asn Asn Gly Val Lys Gly
            340                 345                 350

Phe Ser Tyr Leu Asp Gly Asp Asn Thr Trp Leu Gly Arg Thr Ile Ser
        355                 360                 365

Thr Ala Ser Arg Ser Gly Tyr Glu Met Leu Lys Val Pro Asn Ala Leu
    370                 375                 380

Thr Asp Asp Arg Ser Lys Pro Thr Gln Gly Gln Thr Ile Val Leu Asn
385                 390                 395                 400

Thr Asp Trp Ser Gly Tyr Ser Gly Ser Phe Ile Asp Tyr Trp Ala Lys
                405                 410                 415

Gly Glu Cys Tyr Arg Ala Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg
            420                 425                 430

Pro Lys Glu Asp Lys Val Trp Trp Thr Ser Asn Ser Ile Val Ser Met
        435                 440                 445

Cys Ser Ser Thr Glu Phe Leu Gly Gln Trp Asn Trp Pro Asp Gly Ala
    450                 455                 460

Lys Ile Glu Tyr Phe Leu
465                 470


<210> SEQ ID NO 41
<211> LENGTH: 567
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 41

Met Ile Ala Ile Ile Val Ile Ala Ile Leu Ala Ala Ala Gly Lys Ser
1               5                   10                  15

Asp Lys Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Thr Gln Val
            20                  25                  30

Asp Thr Ile Leu Glu Lys Asn Val Thr Val Thr His Ser Ile Glu Leu
        35                  40                  45

Leu Glu Asn Gln Lys Glu Glu Arg Phe Cys Lys Ile Leu Asn Lys Ala
    50                  55                  60

Pro Leu Asp Leu Arg Glu Cys Thr Ile Glu Gly Trp Ile Leu Gly Asn
65                  70                  75                  80

Pro Gln Cys Asp Leu Leu Leu Gly Asp Gln Ser Trp Ser Tyr Ile Val
                85                  90                  95

Glu Arg Pro Thr Ala Gln Asn Gly Ile Cys Tyr Pro Gly Thr Leu Asn
            100                 105                 110

Glu Val Glu Glu Leu Arg Ala Leu Ile Gly Ser Gly Glu Arg Val Glu
        115                 120                 125

Arg Phe Glu Met Phe Pro Gln Ser Thr Trp Gln Gly Val Asp Thr Asn
    130                 135                 140

Ser Gly Thr Thr Arg Ser Cys Pro Tyr Ser Thr Gly Asp Pro Ser Phe
145                 150                 155                 160

Tyr Arg Asn Leu Leu Trp Ile Ile Lys Thr Lys Thr Ala Glu Tyr Pro
                165                 170                 175

Val Ile Lys Gly Ile Tyr Asn Asn Thr Gly Thr Gln Pro Ile Leu Tyr
            180                 185                 190

Phe Trp Gly Val His His Pro Pro Asn Thr Asp Glu Gln Asp Thr Leu
        195                 200                 205

Tyr Gly Ser Gly Asp Arg Tyr Val Arg Met Gly Thr Glu Ser Met Asn
    210                 215                 220

Phe Ala Lys Ser Pro Glu Ile Ala Ala Arg Pro Ala Val Asn Gly Gln
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225                 230                 235                 240

Arg Gly Arg Ile Asp Tyr Tyr Trp Ser Val Leu Lys Pro Gly Glu Thr
                245                 250                 255

Leu Asn Val Glu Ser Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Tyr
            260                 265                 270

Lys Phe Val Asn Thr Asn Ser Lys Gly Ala Val Phe Arg Ser Asp Leu
        275                 280                 285

Pro Ile Glu Asn Cys Asp Ala Thr Cys Gln Thr Ile Ala Gly Val Leu
    290                 295                 300

Arg Thr Asn Lys Thr Phe Gln Asn Val Ser Pro Leu Trp Ile Gly Glu
305                 310                 315                 320

Cys Pro Lys Tyr Val Lys Ser Glu Ser Leu Arg Leu Ala Thr Gly Leu
                325                 330                 335

Arg Asn Val Pro Gln Ile Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala
            340                 345                 350

Gly Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly
        355                 360                 365

Tyr His His Glu Asn Ser Gln Gly Ser Gly Tyr Ala Ala Asp Arg Glu
    370                 375                 380

Ser Thr Gln Lys Ala Val Asn Arg Ile Thr Asn Lys Val Asn Ser Ile
385                 390                 395                 400

Ile Asn Lys Met Asn Thr Gln Phe Glu Ala Val Asp His Glu Phe Ser
                405                 410                 415

Asn Leu Glu Arg Arg Ile Asp Asn Leu Asn Lys Arg Met Gln Asp Gly
            420                 425                 430

Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu
        435                 440                 445

Asn Glu Arg Thr Leu Asp Met His Asp Ala Asn Val Lys Asn Leu His
    450                 455                 460

Glu Lys Val Lys Ser Gln Leu Arg Asp Asn Ala Asn Asp Leu Gly Asn
465                 470                 475                 480

Gly Cys Phe Glu Phe Trp His Lys Cys Asp Asn Glu Cys Ile Glu Ser
                485                 490                 495

Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Gln Thr Glu Ser Lys
            500                 505                 510

Leu Asn Arg Leu Lys Ile Glu Ser Val Lys Leu Glu Asn Leu Gly Val
        515                 520                 525

Tyr Gln Ile Leu Ala Ile Tyr Ser Thr Val Ser Ser Ser Leu Val Leu
    530                 535                 540

Val Gly Leu Ile Met Ala Met Gly Leu Trp Met Cys Ser Asn Gly Ser
545                 550                 555                 560

Met Gln Cys Asn Val Cys Ile
                565


<210> SEQ ID NO 42
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 42

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Met Val
1               5                   10                  15

Val Gly Ile Ile Ser Leu Met Leu Gln Ile Gly Asn Ile Ile Ser Ile
            20                  25                  30

Trp Val Ser His Ile Ile Gln Thr Gly His Pro Asn Gln Pro Gly Pro
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        35                  40                  45

Cys Asn Gln Ser Ile Asn Phe Tyr Thr Glu Gln Ala Ala Ala Ser Val
    50                  55                  60

Thr Leu Ala Gly Asn Ser Ser Leu Cys Pro Ile Ser Gly Trp Ala Ile
65                  70                  75                  80

Tyr Ser Lys Asp Asn Ser Ile Arg Ile Gly Ser Lys Gly Asp Val Phe
                85                  90                  95

Val Met Arg Glu Pro Phe Val Ser Cys Ser His Leu Glu Cys Arg Thr
            100                 105                 110

Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His Ser Asn Gly
        115                 120                 125

Thr Val Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser Cys Pro Val
    130                 135                 140

Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser Val Ala Trp
145                 150                 155                 160

Ser Ala Ser Ala Cys His Asp Gly Ile Ser Trp Leu Thr Ile Gly Ile
                165                 170                 175

Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr Asn Gly Ile
            180                 185                 190

Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Asn Ile Leu Arg Thr Gln
        195                 200                 205

Glu Ser Glu Cys Ala Cys Val Asn Gly Ser Cys Phe Thr Val Met Thr
    210                 215                 220

Asp Gly Pro Ser Asn Glu Gln Ala Ser Tyr Lys Ile Phe Lys Ile Glu
225                 230                 235                 240

Lys Gly Lys Val Val Lys Ser Val Glu Leu Asn Ala Pro Asn Tyr His
                245                 250                 255

Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Ala Gly Glu Ile Thr Cys Val
            260                 265                 270

Cys Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val Ser Phe Asn
        275                 280                 285

Gln Asn Leu Glu Tyr Gln Ile Gly Tyr Ile Cys Ser Gly Val Phe Gly
    290                 295                 300

Asp Ser Pro Arg Pro Asn Asp Gly Thr Gly Ser Cys Gly Pro Val Ser
305                 310                 315                 320

Leu Asn Gly Glu Tyr Gly Val Lys Gly Phe Ser Phe Lys Tyr Gly Asp
                325                 330                 335

Gly Val Trp Ile Gly Arg Thr Lys Ser Thr Ser Ser Arg Ser Gly Phe
            340                 345                 350

Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Glu Thr Asp Ser Asn Phe
        355                 360                 365

Ser Leu Lys Gln Asp Ile Ile Ala Ile Thr Asp Trp Ser Gly Tyr Ser
    370                 375                 380

Gly Ser Phe Val Gln His Pro Glu Leu Thr Gly Leu Asn Cys Met Arg
385                 390                 395                 400

Pro Cys Phe Trp Val Glu Leu Ile Arg Gly Arg Pro Lys Glu Lys Thr
                405                 410                 415

Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val Asn Ser Asp
            420                 425                 430

Thr Val Gly Trp Ser Trp Pro Asp Gly Ala Glu Val Pro Phe Thr Ile
        435                 440                 445

Asp Lys
    450
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<210> SEQ ID NO 43
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 43

Met Ile Ala Ile Ile Ile Leu Ala Ile Val Val Ser Thr Ser Lys Ser
1 5 10 15

Asp Arg Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Thr Gln Val
20 25 30

Asp Thr Ile Leu Glu Lys Asn Val Thr Val Thr His Ser Val Glu Leu
35 40 45

Leu Glu Asn Gln Lys Glu Asn Arg Phe Cys Arg Val Leu Asn Lys Ala
50 55 60

Pro Leu Asp Leu Met Asp Cys Thr Thr Glu Gly Trp Ile Leu Gly Asn
65 70 75 80

Pro Arg Cys Asp Asn Leu Leu Gly Asp Gln Ser Trp Ser Tyr Ile Val
85 90 95

Glu Arg Pro Asp Ala Gln Asn Gly Ile Cys Tyr Pro Gly Val Leu Lys
100 105 110

Glu Thr Glu Glu Leu Lys Ala Leu Ile Gly Ser Ile Asp Ser Ile Gln
115 120 125

Arg Phe Glu Met Phe Pro Lys Ser Thr Trp Thr Gly Val Asp Thr Asn
130 135 140

Ser Gly Val Thr Ser Ala Cys Pro Tyr Asn Gly Glu Ser Ser Phe Tyr
145 150 155 160

Arg Asn Leu Leu Trp Ile Ile Lys Ile Arg Ser Asp Pro Tyr Ser Leu
165 170 175

Ile Lys Gly Thr Tyr Thr Asn Thr Gly Ser Gln Pro Ile Leu Tyr Phe
180 185 190

Trp Gly Val His His Pro Pro Asp Glu Val Glu Gln Ala Asn Leu Tyr
195 200 205

Gly Ile Gly Thr Arg Tyr Val Arg Met Gly Thr Glu Ser Met Asn Phe
210 215 220

Ala Lys Gly Pro Glu Ile Ala Gly Arg Pro Pro Ala Asn Gly Gln Arg
225 230 235 240

Gly Arg Ile Asp Tyr Tyr Trp Ser Val Leu Lys Pro Gly Glu Thr Leu
245 250 255

Asn Val Glu Ser Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Tyr Lys
260 265 270

Phe Thr Ser Ser Arg Asn Lys Gly Ala Ile Phe Lys Ser Asp Leu Pro
275 280 285

Ile Glu Asn Cys Asp Ala Val Cys Gln Thr Leu Ala Gly Ala Ile Asn
290 295 300

Thr Asn Lys Thr Phe Gln Asn Ile Ser Pro Val Trp Ile Gly Glu Cys
305 310 315 320

Pro Lys Tyr Val Lys Ser Lys Ser Leu Lys Leu Ala Thr Gly Leu Arg
325 330 335

Asn Val Pro Gln Ala Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly
340 345 350

Phe Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr
355 360 365

His His Glu Asn Ser Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser
370 375 380

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Thr Gln Lys Ala Ile Asp Gly Ile Thr Asn Lys Val Asn Ser Ile Ile
385                 390                 395                 400

Asp Lys Met Asn Thr Gln Phe Glu Ala Val Glu His Glu Phe Ser Ser
                405                 410                 415

Leu Glu Arg Arg Ile Gly Asn Leu Asn Lys Arg Met Glu Asp Gly Phe
            420                 425                 430

Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn
        435                 440                 445

Glu Arg Thr Leu Asp Met His Asp Ala Asn Val Lys Asn Leu His Glu
    450                 455                 460

Lys Val Lys Ser Gln Leu Arg Asp Asn Ala Lys Asp Leu Gly Asn Gly
465                 470                 475                 480

Cys Phe Glu Phe Trp His Lys Cys Asp Asn Glu Cys Ile Asn Ser Val
                485                 490                 495

Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Gln Glu Glu Ser Arg Leu
            500                 505                 510

Asn Arg Gln Glu Ile Lys Ser Val Met Leu Glu Asn Leu Gly Val Tyr
        515                 520                 525

Gln Ile Leu Ala Ile Tyr Ser Thr Val Ser Ser Ser Leu Val Leu Val
    530                 535                 540

Gly Leu Ile Ile Ala Met Gly Leu Trp Met Cys Ser Asn Gly Ser Met
545                 550                 555                 560

Gln Cys Lys Ile Cys Ile
                565


<210> SEQ ID NO 44
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 44

Met Asn Pro Asn Gln Arg Ile Ile Thr Ile Gly Ser Val Ser Leu Thr
1               5                   10                  15

Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala Ile Leu Ala Thr Thr
            20                  25                  30

Val Thr Leu His Phe Lys Gln Asn Glu Cys Ser Ile Pro Ala Asn Asn
        35                  40                  45

Gln Val Thr Pro Cys Glu Pro Ile Val Ile Glu Arg Asn Ile Thr Glu
    50                  55                  60

Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Pro Glu
65                  70                  75                  80

Val Val Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
                85                  90                  95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
            100                 105                 110

Asp Ile Trp Ile Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Ser Lys
        115                 120                 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Asp Asn Lys His
    130                 135                 140

Ser Asn Gly Thr Ile His Asp Arg Ile Pro His Arg Thr Leu Leu Met
145                 150                 155                 160

Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Ile
                165                 170                 175

Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
            180                 185                 190
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Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Ile Tyr Asp
        195                 200                 205

Gly Met Leu Ile Asp Ser Ile Gly Ser Trp Ser Gln Asn Ile Leu Arg
    210                 215                 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Ser Gly Thr Cys Thr Val Val
225                 230                 235                 240

Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe
                245                 250                 255

Ile Arg Glu Gly Lys Ile Val His Ile Ser Pro Leu Ser Gly Ser Ala
            260                 265                 270

Gln His Val Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asn Val Arg
        275                 280                 285

Cys Val Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp
    290                 295                 300

Ile Asn Met Ala Asp Tyr Ser Ile Asp Ser Ser Tyr Val Cys Ser Gly
305                 310                 315                 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Ser Ser Asn
                325                 330                 335

Cys Arg Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp
            340                 345                 350

Ala Phe Asp Asn Gly Asn Asp Val Trp Met Gly Arg Thr Ile Ser Lys
        355                 360                 365

Asp Ser Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Ala
    370                 375                 380

Ile Ala Asn Ser Lys Ser Gln Thr Asn Arg Gln Val Ile Val Asp Asn
385                 390                 395                 400

Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Ser Lys Gly
                405                 410                 415

Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Gln
            420                 425                 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
        435                 440                 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
    450                 455                 460

Asp Phe Met Pro Ile
465


<210> SEQ ID NO 45
<211> LENGTH: 1464
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 45

agcaaaagca gggtgatcga gaatgaatcc aaatcagaaa ctatttgcat tatctggagt     60

ggcaatagca cttagtgtac tgaacttatt gataggaatc tcaaacgtcg gattgaacgt    120

atctctacat ctaaaggaaa aaggacccaa acaggaggag aatttaacat gcacgaccat    180

taatcaaaac aacactactg tagtagaaaa cacatatgta aataatacaa caataattac    240

caagggaact gatttgaaaa caccaagcta tctgctgttg aacaagagcc tgtgcaatgt    300

tgaagggtgg gtcgtgatag caaaagacaa tgcagtaaga tttggggaaa gtgaacaaat    360

cattgttacc agggagccat atgtatcatg cgacccaaca ggatgcaaaa tgtatgcctt    420

gcaccaaggg actaccatta ggaacaaaca ttcaaatgga acgattcatg acagaacagc    480

tttcagaggt ctcatctcca ctccattggg cactccacca accgtaagta acagtgactt    540
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tatgtgtgtt ggatggtcaa gcacaacttg ccatgatggg attgctagga tgactatctg 600

tatacaagga aataatgaca atgctacagc aacggtttat tacaacagaa ggctgaccac 660

taccattaag acctgggcca gaaacattct gaggactcaa gaatcagaat gtgtgtgcca 720

caatggcaca tgtgcagttg taatgaccga cggatcggct agtagtcaag cctatacaaa 780

agtaatgtat ttccacaagg gattagtagt taaggaggag gagttaaggg gatcagccag 840

acatattgag gaatgctcct gttatggaca caatcaaaag gtgacctgtg tgtgcagaga 900

taactggcag ggagcaaaca ggcctattat agaaattgat atgagcacat tggagcacac 960

aagtagatac gtgtgcactg gaattctcac agacaccagc agacctgggg acaaatctag 1020

tggtgattgt tccaatccaa taactgggag tcccggcgtt ccgggagtga agggattcgg 1080

gtttctaaat ggggataaca catggcttgg taggaccatc agccccagat caagaagtgg 1140

attcgaaatg ttgaaaatac ctaatgcagg tactgatccc aattctagaa tagcagaacg 1200

acaggaaatt gtcgacaata acaattggtc aggctattcc ggaagcttta ttgactattg 1260

gaatgataac agtgaatgct acaatccatg cttttacgta gagttaatta gaggaagacc 1320

cgaagaggct aaatacgtat ggtgggcaag taacagtcta attgccctat gtggaagccc 1380

attcccagtt gggtctggtt ccttccccga tggggcacaa atccaatact tttcgtaaaa 1440

tgcaaaaaca cccttgtttc tact 1464

<210> SEQ ID NO 46
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Influenza A virus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(39)

<400> SEQUENCE: 46

cct caa aga gag aga aga aga aaa aag aga gga tta ttt 39
Pro Gln Arg Glu Arg Arg Arg Lys Lys Arg Gly Leu Phe
1 5 10

<210> SEQ ID NO 47
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Influenza A virus

<400> SEQUENCE: 47

Pro Gln Arg Glu Arg Arg Arg Lys Lys Arg Gly Leu Phe
1 5 10

<210> SEQ ID NO 48
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(15)

<400> SEQUENCE: 48

cca aag ggg aga ggc 15
Pro Lys Gly Arg Gly
1 5

<210> SEQ ID NO 49
<211> LENGTH: 5
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 49

Pro Lys Gly Arg Gly
1 5

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Pro Lys Thr Arg Gly
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Pro Lys Pro Arg Gly
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<400> SEQUENCE: 54

Arg Arg Lys Lys
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cct caa aga gag act cga gga tta ttt 27
Pro Gln Arg Glu Thr Arg Gly Leu Phe
1 5

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<211> LENGTH: 9
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<400> SEQUENCE: 56

Pro Gln Arg Glu Thr Arg Gly Leu Phe
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<210> SEQ ID NO 57
<211> LENGTH: 21
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<400> SEQUENCE: 57

cca aag agg agg agg aga ggc 21
Pro Lys Arg Arg Arg Arg Gly
1 5

<210> SEQ ID NO 58
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<400> SEQUENCE: 58

Pro Lys Arg Arg Arg Arg Gly
1 5

What is claimed is:

1. A 6:2 reassortant influenza virus, wherein said virus comprises 6 internal genome segments from one or more donor viruses and 2 surface antigen genome segments, wherein the surface antigen genome segments encode an HA and a NA polypeptide, wherein the surface antigen genome segment that encodes the HA polypeptide produces a polypeptide comprising the amino acid sequence of SEQ ID NO:41.

2. The 6:2 reassortant influenza virus of claim **1**, wherein the one or more donor viruses comprises one or more of the following phenotypes: temperature-sensitive, cold-adapted, or attenuated.

3. The 6:2 reassortant influenza virus of claim **1**, wherein the one or more donor viruses are PR8.

4. The 6:2 reassortant influenza virus of claim **1**, wherein the one or more donor viruses are A/Leningrad/17.

5. An immunogenic vaccine composition comprising an immunologically effective amount of the reassortant influenza virus of claim **1**.

6. An immunogenic vaccine composition comprising an immunologically effective amount of the reassortant influenza virus of claim **2**.

7. A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the

individual an immunologically effective amount of the reassortant influenza virus of claim **1** in a physiologically effective carrier.

8. A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the reassortant influenza virus of claim **2** in a physiologically effective carrier.

9. A method of prophylactic or therapeutic treatment of a viral infection in a subject, the method comprising: administering to the subject, the virus of claim **1** in an amount effective to produce an immunogenic response against the viral infection.

10. The method of claim **7**, wherein said virus is killed or inactivated.

11. The method of claim **9**, wherein said virus is killed or inactivated.

12. The method of claim **9**, wherein the subject is a human.

13. A live attenuated influenza vaccine comprising the composition of claim **5**.

14. A live attenuated influenza vaccine comprising the composition of claim **6**.

15. A method for producing an influenza virus in cell culture, the method comprising:

i) introducing into a population of host cells, which population of host cells is capable of supporting replication of influenza virus, a plurality of vectors comprising nucleotide sequences corresponding to:

(a) at least 6 internal genome segments of a first influenza strain, and, at least one genome segment encoding an HA surface antigen polypeptide wherein the surface antigen polypeptide comprises the amino acid sequence of SEQ ID NO: 41; or

(b) at least 6 internal genome segments of a first influenza strain and which influenza strain comprises one or more phenotypic attributes selected from the group consisting of: attenuated, cold adapted and temperature sensitive; and at least one genome segment encoding an HA surface antigen wherein the surface antigen polypeptide comprises the amino acid sequence of SEQ ID NO: 41,

ii) culturing the population of host cells at a temperature less than or equal to 35° C.; and,

iii) recovering an influenza virus.

16. An immunogenic composition comprising an immunologically effective amount of the influenza virus produced by the method of claim **15**.

17. A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual an immunologically effective amount of the influenza virus produced by the method of claim **15** in a physiologically effective carrier.

18. A method for stimulating the immune system of an individual to produce a protective immune response against influenza virus, the method comprising administering to the individual the immunogenic composition of claim **16**.

19. A live attenuated influenza vaccine comprising the immunogenic composition of claim **16**.

20. A split virus or killed virus vaccine comprising the immunogenic composition of claim **16**.

21. The 6:2 reassortant virus of claim **1**, wherein the one or more donor viruses are A/Ann Arbor/6/60.

22. The 6:2 reassortant virus of claim **1**, wherein the one or more donor viruses are other than A/Ann Arbor/6/60.

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